

REPRODUCTION
IN
DOMESTIC ANIMALS

REPRODUCTION IN DOMESTIC ANIMALS

Edited by

H. H. COLE and P. T. CUPPS

University of California, Davis, California

Volume 1



1959

ACADEMIC PRESS, New York and London

Copyright©, 1959, by Academic Press Inc.

ALL RIGHTS RESERVED

NO PART OF THIS BOOK MAY BE REPRODUCED IN ANY FORM,
BY PHOTOSTAT, MICROFILM, OR ANY OTHER MEANS,
WITHOUT WRITTEN PERMISSION FROM THE PUBLISHERS.

ACADEMIC PRESS INC.

111 FIFTH AVENUE

NEW YORK 3, N. Y.

United Kingdom Edition

Published by

ACADEMIC PRESS INC. (LONDON) LTD.

40 PALL MALL, LONDON SW 1

Library of Congress Catalog Card Number 59-7678

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

- A. C. ANDERSEN, *School of Veterinary Medicine, University of California, Davis, California*
- C. R. AUSTIN, *The National Institute for Medical Research, Medical Research Council, London, England*
- VICTOR R. BERLINER, *Division of Endocrinology, Ortho Research Foundation, Raritan, New Jersey*
- J. M. BODA, *Department of Animal Husbandry, University of California, Davis, California*
- HUBERT R. CATCHPOLE, *Department of Pathology, University of Illinois, College of Medicine, Chicago, Illinois*
- M. T. CLEGG, *Department of Animal Husbandry, University of California, Davis, California*
- C. W. EMMENS, *Department of Veterinary Physiology, The University of Sydney, Sydney, Australia*
- WILLIAM F. GANONG, *Department of Physiology, School of Medicine, University of California, Berkeley, California*
- WILLIAM HANSEL, *Department of Animal Husbandry, Cornell University, Ithaca, New York*
- ELMER B. HARVEY, *Department of Zoology and Entomology, Montana State College, Bozeman, Montana*
- LOGAN M. JULIAN, *School of Veterinary Medicine, University of California, Davis, California*
- JOSEPH METTES, *Department of Physiology and Pharmacology, Michigan State University, East Lansing, Michigan*
- T. J. ROBINSON, *Department of Animal Husbandry, The University of Sydney, Sydney, Australia*
- MIRIAM E. SIMPSON, *The Institute of Experimental Biology and Department of Anatomy, University of California, Berkeley, California*
- CHARLES W. TURNER, *Department of Dairy Husbandry, University of Missouri, Columbia, Missouri*
- WALTER S. TYLER, *School of Veterinary Medicine, University of California, Davis, California*
- LEMON J. WELLS, *Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota*
- ELOISE WOOTEN, *School of Veterinary Medicine, University of California, Davis, California*

FOREWORD

This treatise utilizes a unique opportunity. During the last few decades an immense body of accurate information on reproductive phenomena in all higher animals has been gleaned. F. H. A. Marshall had the distinction of producing a pioneer account of these phenomena in an era preceding the development of endocrinology.

The immense usefulness of domestic animals to man very quickly focused attention on the physiology of their reproduction. Their study constituted, of course, a very significant part of Marshall's classic treatise, but the detailed study of each of these forms has been the product of only the last twenty or thirty years. In this present volume the results of these studies have been brought together and synthesized.

The primary object of this effort consists in the attempt to bring about a fuller understanding of the complex mechanisms involved in reproduction in order to utilize such an understanding in breeding farm animals. Such an aim can be accomplished solely by sound knowledge of the intricate internal mechanisms (nervous and endocrine) of reproduction and of the external factors (food and environment) which play vital roles here. *The effort to understand these controlling mechanisms is set forth here.* Physiological phenomena are the main concern. Genetics is not treated in detail, although consideration has been given to the influence of heredity on many reproductive phenomena.

Perhaps one of the most striking series of events in the early history of study in this realm was created by three investigations concerning small laboratory animals. The three papers were those of Stockard and Papanicolaou on the guinea pig, of Long and Evans on the rat, and of Edgar Allen on the mouse. They demonstrated that the sequence of steps in the development of the so-called "estrous rhythm" could be clearly shown by the types of cells found free in the vaginal fluid. It appeared, indeed, for a time that the application of the vaginal smear method would be all that was required to segment the stages of the estrous cycle in all animals. Early studies by Hammond in the cow, by McKenzic in the sow, by Andrews and McKenzie in the mare, and by Cole in the cow and ewe did not substantiate this optimism; the beautifully distinct changes seen in the vaginal lochia of small rodents were peculiar for the smaller forms. Only in the dog, as determined by Evans and Cole, was the estrogen level high enough for pronounced vaginal cornification which divulges ovarian changes. We were quickly

forced back to old-fashioned but eminently reliable observations of such matters, for instance, as changes in behavior.

In behavior changes the internal mechanisms, both endocrine and nervous, are, of course, at work. External controlling factors are also becoming known with increasing accuracy. Two domestic species, the ewe and the mare, are seasonal breeders. The reader will find here a discussion of the fact that the sexual season is induced in the ewe by withdrawal of light, and in the mare by increased light, as had been previously found in birds. The role of temperature is also definite, for it may delay the onset of the ewe's sexual season, cooling hastening this event.

The endocrine changes in pregnancy have led, as is well known, to dramatic discoveries, for example, that of the very high hemal titers in female sex hormone in the pregnant mare (subsequently amazingly found in the stallion) and in reliable pregnancy tests. Perhaps nothing is stranger than the similarity between the mare and women in high pregnancy estrogen and gonadotropin titers. In the mare, unlike the situation in women, the gonadotropin appears to be secreted by maternal structures called *endometrial cups* and not by the chorionic tissue of her offspring, although estrogens are secreted by the chorion in both forms. Explanations for the sudden great overproduction of these gonadotropins are not at hand.

The tremendous importance of artificial insemination in animal breeding has served as a stimulus for intensive research on spermatogenesis, on the biochemistry of semen, and on the factors influencing sexual *libido* and sperm production. It is perhaps in this area that some of our knowledge in domestic animals compares favorably with, and in some instances surpasses, that available in laboratory animals.

If this beautiful treatise can thus serve as an impetus for the acquisition of new, necessary, and especially quantitative data, measuring all of these phenomena, its essential purpose will have been abundantly fulfilled.

HERBERT M. EVANS

*Institute of Experimental Biology
University of California, Berkeley, California
March, 1959*

PREFACE

Designing a book to be useful as a text for advanced undergraduate and graduate students, for research workers in the field of reproduction, and for veterinary clinicians presents an interesting challenge. Beyond furnishing an anatomical background, the first six chapters of this book outline modern concepts of reproductive physiology in mammals. The remaining portions deal more specifically with reproduction in domestic animals; the authors, nevertheless, have not hesitated to draw upon knowledge of these events in laboratory animals where they have been elaborately worked out there. Because of its size, this treatise has been divided into two volumes.

Differences in interpretation are not uncommon; the editors have neither desired nor attempted to harmonize viewpoints. These differences reflect the incompleteness of our knowledge. For instance, follicle stimulating and luteinizing hormones, as purified from anterior lobe tissue, have not been clearly demonstrated in the blood or urine of any species. Some authors have assumed that both are true hormones and actually secreted; others have taken a more cautious position.

Usage of terms has, to some extent, been standardized. Consistent usage of the terms "metestrus," "diestrus," and "anestrus" has been difficult to achieve. Inadequate knowledge of the intimate changes in the reproductive organs when Walter Heape introduced the terms accounts in part for this difficulty; species differences in secretory activity of the ovary during the postestrous interval with resulting variations in the complexity of development of the accessory structures is a second complicating factor. Although agreement on the use of these terms would be desirable, the problem is obviously too involved to effect uniformity in the present volumes. Action by appropriate bodies to standardize usage of these terms would be desirable.

The editors take this opportunity to express their appreciation to the authors for preparing their chapters meticulously and promptly. Excellent cooperation by the authors is evidenced in that scarcely more than a year elapsed between receipt of the first manuscript and publication of the book.

For many years reproductive physiology has been greatly enriched by the signal contributions made by Dr. Herbert M. Evans and his colleagues. Many, including the senior editor, have benefited from a sojourn in the inspirational atmosphere of his laboratory. Our thanks are due him as author of the Foreword.

We should like to express our thanks to Miss Lee Doyle and Mrs. June Law for assistance in the preparation of the subject index.

Finally, the friendly helpfulness of the Academic Press staff has contributed toward making editing of these volumes a pleasant task.

H. H. COLE

P. T. CUPPS

Davis, California

March, 1959

CONTENTS

LIST OF CONTRIBUTORS	v
FOREWORD	vii
PREFACE	ix
CONTENTS OF VOLUME II	xv
1. Anatomy of Female Reproductive Organs	1
LEMEN J. WELLS	
I General Introduction	1
II Development of Female Reproductive Organs	2
III Experimental Studies of the Role of the Developing Gonads in Sex Differentiation	13
IV Anatomy of the Reproductive Organs after Birth	18
References	23
2. Anatomy of the Male Reproductive Organs	29
LOGAN M. JULIAN AND WALTER S. TYLER	
I Introduction	29
II Development of Male Reproductive Organs	30
III Postnatal Anatomy of the Reproductive Organs	31
References	55
3. Role of Anterior Pituitary Gonadotropins in Reproductive Processes	59
MIRIAM E. SIMPSON	
I Dependence of the Gonads upon the Anterior Pituitary	60
II The Pituitary Gonadotropic Complex: FSH and ICSH (LH)	67
III Prolactin as a Member of the Gonadotropic Complex	74
IV Chemical Fractionation of Pituitary Gonadotropins	75
V Species Specificity in Pituitary Gonadotropins	77
VI Regulation of Production and Secretion of Pituitary Gonadotropins	78
VII Hormonal Factors Necessary for Ovulation	81
VIII Hormonal Factors Necessary for Establishment and Maintenance of Pregnancy	83
IX Hormonal Interrelations in Problems of Fertility	84
X Dietary-Hormonal Interrelations in Reproduction	86
XI Gonadotropins in Body Fluids	88
XII Bioassay of Gonadotropins	90
XIII Relation of Pituitary Gonadotropins to Those in Body Fluids	100
References	108

4. Role of Gonadal Hormones in Reproductive Processes 111

C. W. EMMENS

I. Introduction	112
✓II. Androgens	119
✓III. Estrogens	129
✓IV. Progesterone	137
✓V. The Estrous Cycle	140
VI. Relaxin	146
References	147

5. Role of Thyroid, Adrenal, and Posterior Pituitary Hormones in Reproductive Processes 155

CHARLES W. TURNER

I. Introduction	156
II. Role of Thyroid Gland and Thyroxine in Reproduction	157
III. Role of the Adrenal Glands and Their Hormones in Reproduction	172
IV. Role of Oxytocin in Reproduction	177
References	180

6. Role of the Nervous System in Reproductive Processes 185

WILLIAM F. GANONG

I. Introduction	185
II. Regulation of Pituitary Gonadotropin Secretion by the Nervous System	186
III. Mating Behavior	203
IV. The Onset of Puberty	211
V. Parturition-Neural Factors	214
VI. Lactation-Neural Factors	215
References	216

7. The Estrous Cycle of the Cow ✓ 223

WILLIAM HANSEL

I. Introduction	224
II. The Nature of the Cycle	224
III. Changes in Reproductive and Endocrine Organs during the Cycle	229
IV. Effects of Various Hormones on the Reproductive Tract	243
V. Changes in Other Endocrine Glands during the Estrous Cycle ..	247
VI. Methods of Altering the Cycle	250
VII. Ovarian Hormone Levels in Blood and Excreta during the Estrous Cycle	253
VIII. The Mechanism of Ovulation in the Cow	254
References	260

8. The Estrous Cycle of the Mare	267
VICTOR R BERLINER	
I The Breeding Season of Mares	267
II The Pattern of the Estrous Cycle of the Mare	271
III Physiological and Histological Changes in the Reproductive System	277
IV The Behavioral Pattern of the Cyclic Mare	284
V Adaptation of the Breeding Program to Cyclic Events	285
References	287
9. The Estrous Cycle of the Ewe and Doe	291
T J ROBINSON	
Part I The Ewe	292
I Introduction	292
II The Sexual Season	292
III The Estrous Cycle	295
IV Cyclic Changes in the Reproductive Organs	296
V Endocrine Control of the Cycle	305
VI Artificial Control of the Cycle	324
Part II The Doe	328
VII Introduction	328
VIII Characteristics of the Cycle	328
References	329
10. The Estrous Cycle of the Sow	335
J M BODA	
I Introduction	335
II Prepuberal Development	336
III The Attainment of Puberty	337
IV The Estrous Cycle	338
References	355
11. The Estrous Cycle of the Dog	359
A C ANDERSEN AND ELOISE WOOTEN	
I Introduction	359
II Development of the Genital Organs to Puberty	360
III The Estrous Cycle	364
IV Factors Influencing the Estrous Cycle	384
V Breeding Whelping Lactation, and Puppy Production	392
References	393
12. Fertilization and Development of the Egg	399
C R AUSTIN	
I Maturation, Ovulation, and Transport of Eggs	400
II Transport of Spermatozoa	406

III. Events Leading to Fertilization	408
IV. Fertilization	410
V. Cleavage	418
VI. Maintenance of the Early Embryo	427
References	431

13. Implantation, Development of the Fetus, and Fetal Membranes

ELMER B. HARVEY

I. Introduction	433
II. Implantation	436
III. Yolk Sac and Vitellochorion	438
IV. Amnion	442
V. Chorion, Allantois, Allantochorion	445
VI. Chorioallantoic Placenta	451
VII. Aging and Fetal Development	461
References	466

14. Endocrine Mechanisms During Pregnancy \.....

HUBERT R. CATCHPOLE

I. Introduction	470
II. General Endocrine Mechanisms in Pregnancy	471
III. Special Aspects of Pregnancy in Domestic Animals	497
References	501

15. Factors Affecting Gestation Length and Parturition

M. T. CLEGG

I. Introduction	509
II. Factors Affecting Length of Gestation	509
III. Prolonged Gestation	519
IV. Parturition	522
V. The Initiation of Parturition	526
References	533

16. Mammary Growth and Lactation

JOSEPH MEITES

I. Introduction	539
II. Hormonal Requirements for Udder Growth	540
III. Hormonal Requirements for Lactation	546
References	589

AUTHOR INDEX	595
-------------------------	-----

SUBJECT INDEX	626
--------------------------	-----

REPRODUCTION IN DOMESTIC ANIMALS

VOLUME II

Spermatogenesis and Morphology of the Spermatozoon

R. ORTAVANT

Biochemistry of Semen and Secretions of Male Accessory Organs

T. MANN

Libido in the Male

L. E. ROWSON

Techniques of Collection, Dilution, and Storage of Semen

L. E. ROWSON

Insemination Techniques

JOHN O. ALMQUIST

Nutrition and Reproduction in Domestic Animals

JOHANNES MOUSTGAARD

Environmental Factors Other Than Nutrition, Affecting Reproduction

M. T. CLEGG AND W. F. CANONG

Anatomical and Physiological Factors Affecting Fertility in Domestic Animals

JOHN P. MIXNER

Infectious Diseases Influencing Reproduction

GEORGE H. HART AND JOHN W. OSEBOLD

Reproduction in the Domestic Fowl: Physiology of the Female

A. VAN TIENHOVEN

Reproduction in the Domestic Fowl: Physiology of the Male

F. W. LORENZ

Anatomy of Female Reproductive Organs

LEWIS J. WELLS

	Page
I General Introduction	1
II Development of Female Reproductive Organs	2
A Ovary	2
B Uterine Tubes Uterus and Vagina	11
III Experimental Studies of the Role of the Developing Gonads in Sex Differentiation	13
A Preview	13
B Observations in a Nonplacental Mammal	15
C Observations in Placental Mammals	15
1 Males	16
2 Females	16
D Overview	17
IV Anatomy of the Reproductive Organs after Birth	18
A Ovary	18
B Uterine Tubes	20
C Uterus	21
D Vagina	23
References	23

I GENERAL INTRODUCTION

The following account of the female reproductive tract deals with mammals, and is based in part on domestic animals. It reflects knowledge recorded in the period from 1950 to 1957, but includes older observations as well. It has illustrations obtained from the writer's dissections of fetal calves.*

Brief remarks about the 'nuclear chromatin body' in somatic cells may be useful with respect to the problem of recognizing the sex of individuals in doubtful cases. The finding of this body in a majority of the examined cells is regarded as evidence that the individual is a genetic female with a pair of sex chromosomes, XX, if this body is in only a few of the cells, the individual is probably a genetic male with the XY sex chromosomes (cf references 81 and 153).

The incidence of this body seems to have value in diagnosing the genetic sex (a) in cases of ovarian agenesis or dysgenesis (69, 87, 96),

* The fetuses for this purpose were collected in person at the Birtusch Packing Company, St. Paul, Minnesota through the courtesy of Mr. R. E. Birtusch and Dr. C. M. Crouch.

(b) in embryos too young ("indifferent stage") to show the morphological differentiation of sex (39, 40, 104, 153); and (c) in human fetuses *in utero* [by examining the cells in the amniotic fluid (81)].

The bibliography has several references to works which in the text are not discussed in detail: texts on the anatomy (123) and histology (34, 131) of domestic animals; a treatise on veterinary obstetrics (117); an atlas of fetal and neonatal histology (133); monographs on patterns of reproduction (2) and the physiology of the uterus (116); a short account of the development of the reproductive organs (59); a consideration of the endocrinology of the ovary (132); a review of the morphology of the reproductive tract (31); reviews of cyclic changes in the ovary (12) and in the accessory reproductive organs of the non-pregnant female (32); reviews of the physiology of reproduction (44, 52, 71, 95, 121); studies of dimensions and weights of fetal calves (35, 98, 99, 150) and sheep (43); and a review of the human ovary in pregnancy (97).

II. DEVELOPMENT OF THE FEMALE REPRODUCTIVE ORGANS

Our understanding of this subject has been increased by the availability of a closely graded series of human embryos and of monkey embryos of known age. Accordingly, we are especially indebted to C. H. Heuser (53) for his incomparable techniques in embryos, to C. G. Hartman (51) for mastering the menstrual cycle in the rhesus monkey and then obtaining accurately timed embryos, and to the late G. L. Streeter (129) for placing the human embryos of the Carnegie collection into age groups or developmental horizons.

A. Ovary

The early development of the ovary involves the formation of three structural elements: primordium, gonadal blastema, and primary sex cells. The primordium is a local thickening of the mesothelium (coelomic epithelium), which is lateral to the base of the embryonic mesentery. Here the basement membrane disappears, and the proliferating mesenchymal cells sink into the subjacent mesenchyme. Such migrating cells, together with locally proliferating mesenchyme, comprise the gonadal blastema (47). It is reported that this blastema eventually gives rise to the primary sex cords, to the rete cords or tubes (rete ovarii), and, in a male gonad, to the interstitial cells of the testis (47). As to the primary sex cells, we have recent agreement that in man they do not originate in the gonad itself. One view is that they arise from the entoderm of the hindgut and enter the gonad by migrating along the embryonic mesentery (152). A recent work subscribes to this view

(101). A second and less definite view is that the sex cells arise outside the gonadal blastema (38).

The developing gonad soon acquires a set of primary sex cords from the gonadal blastema [Gruenwald's view (47)], the old view is that the cords arise exclusively from the mesenchymal primordium of the

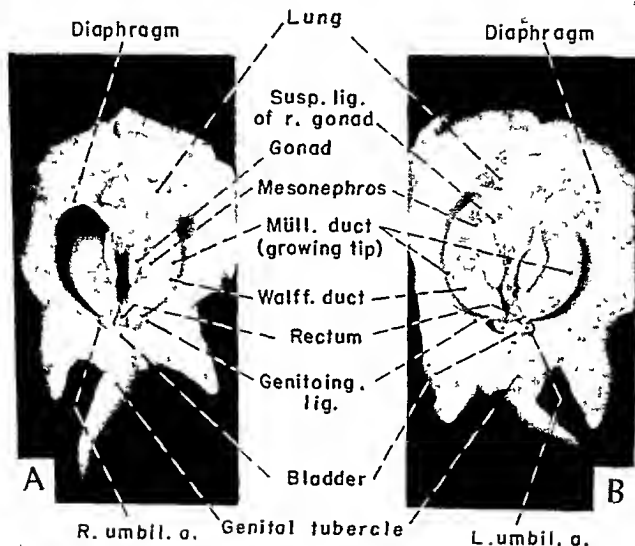


FIG. 1. Ventral aspect of the developing reproductive organs in each of two bovine embryos of 22 mm (crown-rump). The embryos are estimated to be about 40 days of age (150). They are too young to show morphological sex differentiation (indifferent stage). The growing tips of the Mullerian ducts have not yet reached the cloaca. Stained sections of the gonads show primary sex cords (not illustrated) Magnification: $\times 6.1$. (L J Wells, unpublished)

gonad. At the time when these cords appear (Fig. 1), it is not possible to determine whether the gonad is to become an ovary or a testis (the indifferent stage), unless it eventually turns out that the incidence of the nuclear chromatin body in somatic cells is a reliable criterion (153).

The ovarian secondary sex cords appear at a time when a thin layer of connective tissue separates the primary sex cords from the meso-

theum (47). The mesothelium splits into two layers, basal and superficial, the latter lining the coelom. The basal layer gives rise to the secondary sex cords, and in this process, is entirely used up. A similar pattern of development has been reported in the rat (130).

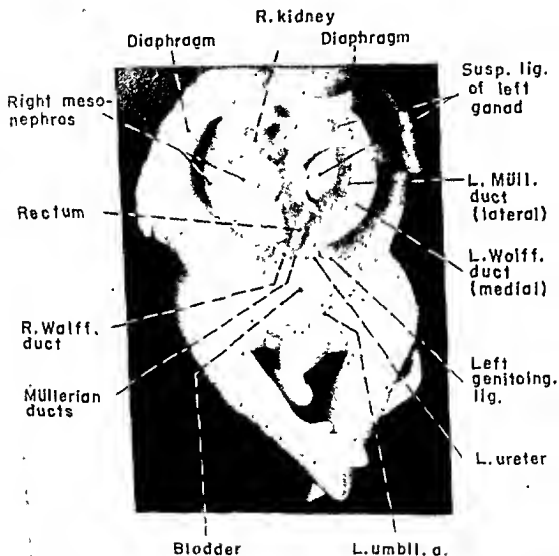


FIG. 2. Developing reproductive organs in a bovine fetus of 40 mm. (about 50 days). The familiar crossing of the two sets of ducts, Wolfian and Mullerian, is more evident in the field of a dissecting microscope than in the photograph presented. The fetus is probably a male. Magnification: $\times 5.5$. (L. J. Wells, unpublished).

In the ovary of the human embryo, Gillman derives the follicular granulosa cells from the mesothelium (38). He also derives the cells of the theca from mesenchyme.

The topography of the developing ovary in man has been depicted

in illustrations obtained from models (61). These pictures fail to show the suspensory ligament of the gonad, a ligament by which the gonad is suspended from the diaphragm. This ligament, a duplication of peritoneum, is shown in Figs. 3 to 8 [see also Fig. 33 in a paper by Wells (144)].

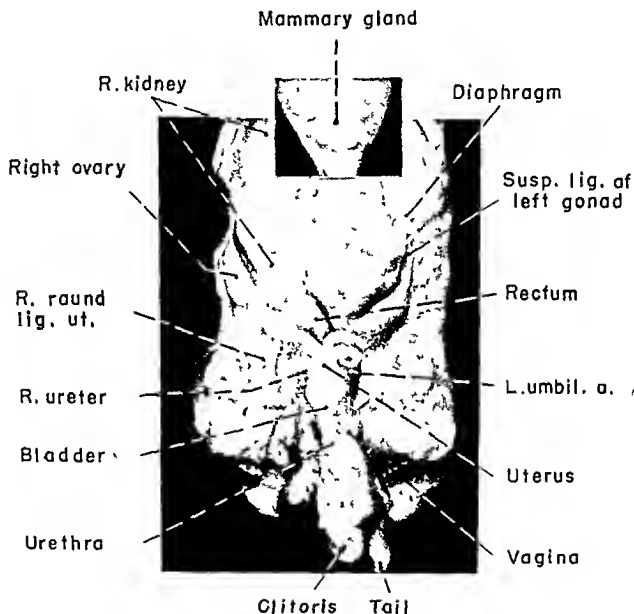


FIG. 3 Ventral aspect of the reproductive organs in a female bovine fetus of 114 mm (70+ days) (150). The mammary gland has been excised, photographed separately, and presented as an insert. Magnification $\times 2.5$ (L. J. Wells, unpublished).

It is said that the mesothelium continues to form new ova during the fetal period of man (118). It would seem that this phenomenon occurs in the fetal monkey, as is suggested by Fig. 9. In any event, there are many primordial follicles in the ovaries of newborn human infants, more than 700,000 according to one set of counts (8).

The ovarian follicles may undergo maturation before birth; and, in man, certain follicles may be multiovular (3). The absence of multinuclear ova in the fetal ovary suggests that the presence of such ova in the postnatal period is due to the absence of maternal gonadotropic

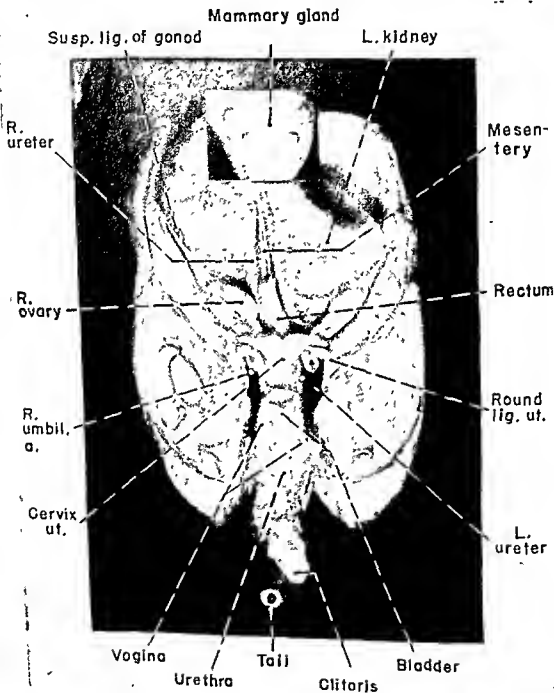


FIG. 4. Ventral view of the reproductive organs in a female bovine fetus of 165 mm. (about 90 days). Magnification: $\times 2$. (L. J. Wells, unpublished.)

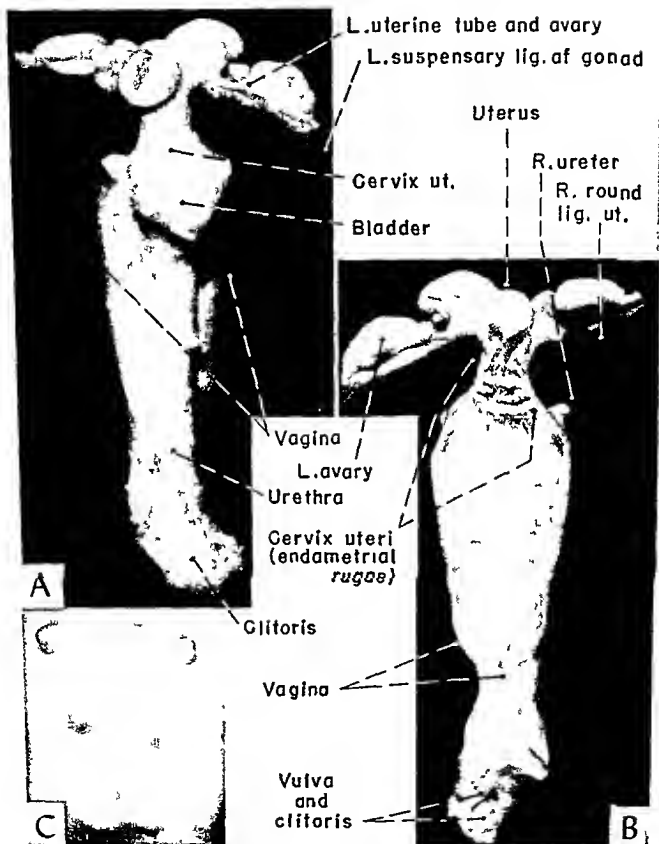


FIG 5 Reproductive tract, A and B, and mammary gland C, from a female bovine fetus of 226 mm (100+ days) (150). The tract is shown in two views, ventral, A, and dorsal, B. The view presented in B was obtained by excising in part the dorsal wall of the vagina and that of the cervix uteri, thus exposing the vaginal cavity and the endometrial rugae in the cervix. The mammary gland shows a super-numerary pair of nipples. Magnification $\times 2.5$ (L. J. Wells, unpublished).

hormones after birth (3). Atretic follicles occur before birth, and one group of workers suggests that this phenomenon points to the stimulation of follicles by gonadotropin during prenatal life (100).

The interstitial cells of the ovary are generally thought to originate from the theca interna of regressing follicles (follicular atresia). It

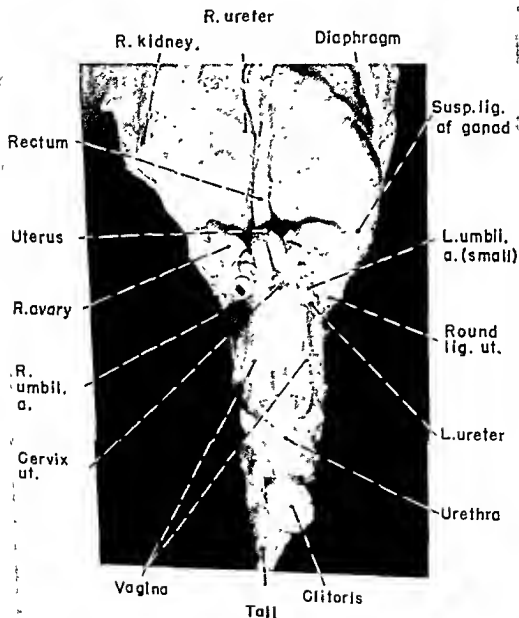


FIG. 6. Ventral view of the reproductive organs in a female bovine fetus of 260 mm. (110+ days) (150). The vaginal cavity and the endometrial rugae in the cervix uteri have been exposed by removing in part the ventral wall of the vagina and that of the cervix. Magnification: $\times 1.2$. (L. J. Wells, unpublished.)

has been reported that the interstitial cells are detectable in a human fetus of $5\frac{1}{2}$ lunar months (118).

The medullary sex cords of the human ovary, the homologues of the seminiferous tubules of the testis, usually disappear prior to birth.

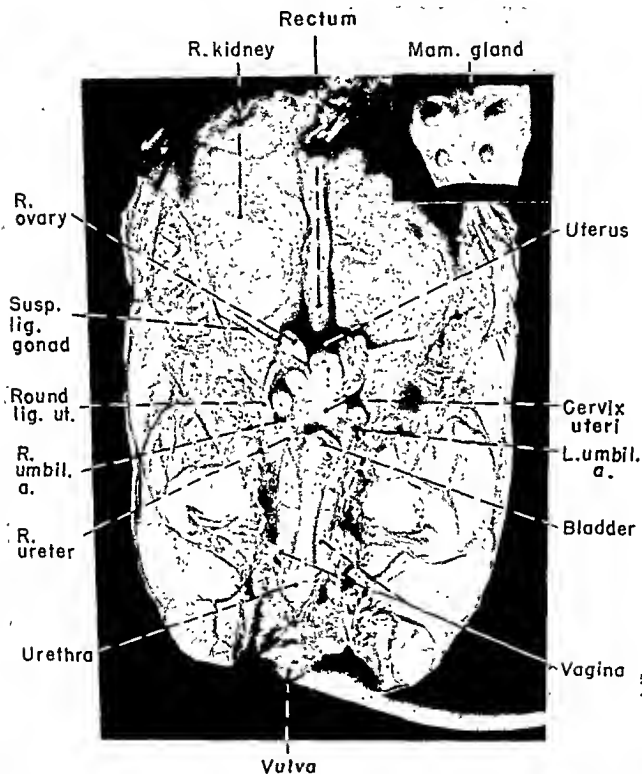


FIG. 7. Ventral aspect of the reproductive tract in a female bovine fetus of 380 mm. (150+ days) (150). The mammary gland has been excised, photographed separately, and presented as an insert. 14/15 actual size. (L. J. Wells, unpublished.)

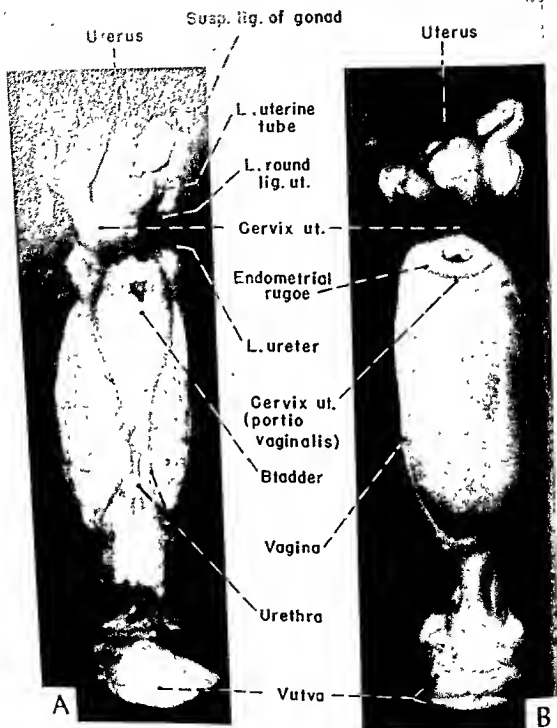


FIG. 8. Reproductive tract from a female bovine fetus of 380 mm. (150+ days) (150). A, ventral view. B, dorsal view of the tract after the ventral wall of the vagina has been excised in part in order to expose the vaginal cavity. Magnification: $\times 2$. (L. J. Wells, unpublished.)

Germ cells are present in all such cords until the latter structures disappear, almost always by the 9th lunar month of gestation (36)

The ponderal growth of the ovaries has been studied in the fetal dog (74) In fetuses up to 40 grams of body weight, the ovaries weigh less than the testes At 440 grams, the reverse is true, the ovaries testes ratio being 2.36 to 1

B Uterine Tubes, Uterus, and Vagina

The uterine tubes, uterus, and possibly the upper portion of the vagina are derivatives of the Mullerian (paramesonephric) ducts At least the lower portion of the vagina, or perhaps all of it, originates from the ventral portion of the cloaca (the urogenital sinus)

The Mullerian ducts are in process of formation in a monkey embryo of 36 days and in human embryos of about 37 days (ovulation age), according to Streeter (129) The first step in this process is a thickening of the mesothelium (coelomic epithelium) at the cephalic end of each mesonephros Then follows an invagination of this mesothelium to produce the ostium of the duct The growing tip of each duct is at first solid (cf Fig 1), and is situated inferiorly (caudally) This tip eventually reaches the urogenital sinus portion of the cloaca and, being solid, ends blindly in the dorsal wall of the urogenital sinus on the Mullerian tubercle (61)

In its downward growth each Mullerian duct follows the course of the mesonephric (Wolffian) duct (cf Figs 1 and 2) In fact, we have evidence that the Wolffian duct induces the formation of the Mullerian duct by acting as an embryonic organizer (45, 46) The evidence is threefold First, the experimental interruption of the Wolffian duct in the chick embryo prevents the Mullerian duct on that side of the body from reaching the cloaca (45) Second, absence of the Mullerian duct in man is associated with absence of the kidney on that side of the body (60), since 1932 we have known that in man renal agenesis may be attributed to retardation in development of the ureter, to embryonic agenesis of the ureter, or to arrest in development of the parent tissue of the ureter, i e, the Wolffian duct (11) The third evidence is that in young embryos the Mullerian and Wolffian ducts are intimately associated with each other, without any intervening basement membrane (46)

In the embryonic region of the future pelvis, the right and left Mullerian ducts fuse with each other to produce the uterovaginal canal (cf Fig 2), the forerunner of the uterus and possibly of the upper part of the vagina (61). It would seem that in man any embryonic induction of Mullerian duct by the Wolffian duct in a female is completed by

stage when male embryos show the beginning of the regression of the Müllerian ducts (60).

In females, the gubernaculi are attached to the body wall in the inguinal region. In males, the gubernaculi, the genito-inguinal ligaments, as shown in Fig. 1, become the round ligaments of the uterus. In males, the gubernaculi or the gubernaculi of the testes (gubernaculi) in males (Fig. 2), after the regression of the Müllerian ducts, are attached to the Wolffian ducts in the region of the epididymides (137).

The developing uterus in man is at first vertically situated, lacking the later anteversion and anteflexion (61). During fetal life the cervix uteri grows more rapidly than the corpus. Up to the 7th lunar month of pregnancy, the length of the human uterus shows a lineal increase (122). Next, the uterus enters a phase of augmented growth which lasts until birth. Then, in the first three postnatal weeks, the uterus loses length until it exhibits essentially the dimensions it would have had if the early lineal increase (rate of growth) had remained unchanged. These observations suggest that placental estrogen is a causative factor in the augmented phase of uterine growth before birth.

In the uteri of 169 newborn infants, 28% showed a secretory type of endometrium, and 5% showed a progestational type which included a decidual reaction (100). These observations point to the stimulating action of the progesterone of pregnancy. The decidual reaction is interpreted by Ober and Bernstein (100) to mean that progesterone crosses the placental membrane and that this hormone itself produces the decidual reaction via the Arthus phenomenon.

The embryology of the vagina is of special interest for two reasons. First, carcinoma of the cervix uteri may originate in the vaginal portion of the cervix (portio vaginalis). Second, a common feature of female pseudohermaphroditism is absence of the lower portion of the vagina, in which case the existing upper portion of the vagina opens caudally into an abnormally persisting embryonic structure, the urogenital sinus. Such a sinus would receive urine via the urethra and menstrual blood via the vagina, as is the case in adult pseudohermaphroditic monkeys (genetic females) which are obtained experimentally by giving testosterone propionate to their mothers during pregnancy (148).

The human vagina, according to one description, has a dual origin, the upper four fifths arising from the uterovaginal canal (fused Müllerian ducts) and the lower one fifth from the urogenital-sinus portion of the embryonic cloaca (70). The primordia of the lower one fifth, called sinovaginal bulbs, are bilateral dorsal evaginations of the urogenital sinus.

A different view has been presented by Meyer (89). According to this view, the Mullerian ducts contribute no more than an original framework of the upper vagina. Eventually the epithelium of this Mullerian framework disappears, and the entire vagina becomes lined by epithelium which migrates upward (cranially) from the urogenital sinus.

Meyer's view is supported by observations in opossums and monkeys, especially in regard to the cellular responses to estrogen. In the opossum, the epithelium of the urogenital sinus migrates upward as far as the cervix uteri (18). But this epithelium is thought to arise, wholly or in part, from the ectodermal portion of the cloacal membrane after the rupture of that membrane. In monkeys special attention is directed to the histogenetic potency of the cloacal region (154). There is no apparent line of histological demarkation separating the proliferative response of the vagina to estrogen from that of the vestibule of the vulva, and, in certain species, from that of skin ("sex skin") lying beyond the vulva.

In line with Meyer's view is the observation that in the human fetus the epithelium of the external os of the cervix uteri degenerates and is shed, producing the condition known as pseudo erosion congenita (61). Meyer's view is confirmed by newer observations (14, 110). The upward extension of epithelium of the urogenital sinus throughout the length of the vagina is completed by the 140 mm stage (14). The "boundary line" of junction of the two original elements of the developing vagina (utero vaginal canal and urogenital sinus) is estimated to lie in the vaginal region of the cervix uteri near the posterior fornix of the vagina (110).

One worker suggests that the Wolffian ducts contribute cells to the developing human vagina (134). This possibility is not supported by other published observations (13). Carr's work in rabbit embryos led him to conclude that in this species the Wolffian ducts do contribute cells to the vagina (21).

III EXPERIMENTAL STUDIES OF THE ROLE OF THE DEVELOPING GONADS IN SEX DIFFERENTIATION

A Preview

Three preliminary considerations should be introduced. First, it is generally agreed that in placental mammals the hormones of pregnancy influence the development and growth of the reproductive organs. The hormones of pregnancy have, however, three possible sites of origin: the pregnant female, the embryo, and the placenta. Hence, the detection of a causal relation between an individual hormone and an in-

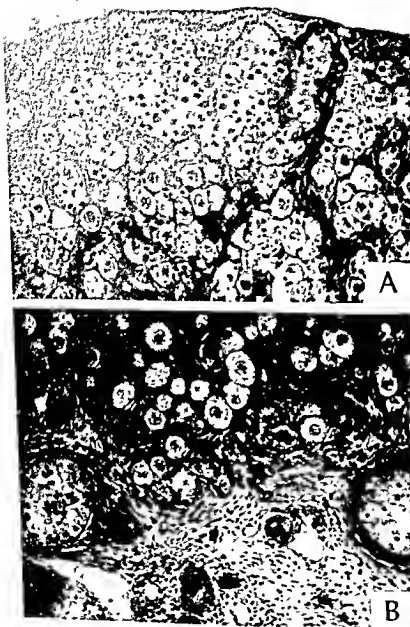


FIG. 9. Sections of the right ovary from a newborn female monkey in which the mother had been subjected to injections of testosterone propionate during the pregnancy. Section A shows the germinal epithelium on the ovarian surface (top), secondary sex cords (two in continuity with the germinal epithelium), and primordial ovarian follicles (bottom). Section B shows the medullary portion of the ovary (bottom), paramedullary part of the cortical portion (top), two large and isolated sex cords (left and right edges of the photomicrograph), and a number of primordial follicles. This right ovary also contains growing follicles (not illustrated). Magnification: $\times 160$. [Reproduced from a paper by Wells and van Wagenen (148), with the permission of the Carnegie Institution of Washington, Department of Embryology, Baltimore, Maryland.]

dividual developing organ involves complications, including the passage of certain hormones through the placental membrane and the nonpassage of other hormones (e.g., "placental barrier")

Second, for 40 years we have had Lillie's theoretical explanation of the "free martin," a sterile bovine female born co-twin with a male (77). The essence of this theory is that testicular androgen from the male twin brings about the free martin condition in the female twin. Despite many attempts to duplicate experimentally the free-martin condition in mammals, none has been successful.

Third, any androgen produced by the fetal testis is not necessarily identical with one of such steroid hormones as androsterone, testosterone, and testosterone propionate. This matter should be kept in mind in considering, for example, the fact that in the rhesus monkey the experimental introduction of testosterone propionate into the maternal circulation during pregnancy may induce in a female fetus the abnormal condition of female pseudohermaphroditism (148). The significance of the ovarian medullary cords (Fig. 9) is not entirely clear.

B Observations in a Nonplacental Mammal

The placenta as a source of hormones has been ruled out by choosing as experimental animal the opossum (*Didelphis*), a nonplacental mammal with a marsupium (90, 91). The young are sexually undifferentiated at birth. By the 20th postnatal day the male shows a few prostatic buds, and the female has Mullerian ducts which have reached the urogenital sinus. When males and females were gonadectomized on the 20th day and permitted to live for several weeks, the development of the reproductive tracts was essentially normal up to the prepubertal stage (subsequent to day 100). This observation suggests that if the developing gonads secrete sex hormones, these hormones are not essential for a continuation of sex differentiation.

The effects of introduced estrogen upon the testes of newborn opossums attract attention (19, 20). In a few cases the testes were converted into ovotestis-like organs, but the induced cortex lacked sex cells. In other cases, the induced cortex contained gonocytes and primordial follicles (20).

The influence of introduced sex hormones upon the developing accessory reproductive organs of newborn opossums of both sexes has been reviewed (20, 91).

C Observations in Placental Mammals

Technical advances have opened up new horizons for investigation in placental mammals. Fetal rats were subjected to castration (138) and

to repeated subcutaneous injections of gonadotropin (139, 142). Fetal rats were deprived of their hypophyses by decapitation (141). A method of castrating fetal rabbits was described (64). In mouse embryos the gonads or hypophyses were destroyed by passing narrow beams of X-rays through the uterine wall, fetal membranes, and particular regions of the fetus (114, 115). In fetal rats the developing reproductive tracts were explanted *in toto*, without gonads, or together with gonads of the opposite sex; sometimes, crystalline steroid hormones were added to the culture medium (112).

1. Males

From work in fetal rats, rabbits, and mice, we have several lines of evidence that the developing testes produce a male sex hormone (androgen) and that this hormone stimulates the development and growth of the male accessory reproductive organs: (a) absence of the testes retards the development and growth of the male accessory organs of reproduction (64-66, 112-114, 140, 143, 145-147); (b) this effect can be prevented, by means of crystalline androgen, in fetal rats (112, 140, 143, 145-147) and rabbits (64-66); (c) it also can be prevented by explanted fetal testes (112); (d) the usual pattern of changes caused by bilateral castration is not induced by unilateral castration in fetal rats (147) and rabbits (64); (e) the changes following bilateral castration are not induced by subjecting normal fetal rats to bilateral adrenalectomy (68).

Although it is possible that the fetal hypophysis cerebri produces a gonadotropin which influences the functioning of the fetal testes, the writer is not aware of such proof. Depriving a fetus of the hypophysis does not induce any major changes in the development of the reproductive tract (66, 113, 145). The latter observation should not be taken to mean, however, that the fetal testes lack the ability to respond to a gonadotropic stimulus. The reverse is actually the case, because injected chorionic gonadotropin increases the number, size, and granularity of the interstitial cells of the fetal testis (139).

2. Females

It is not clear as to whether the fetal ovaries produce sex hormones which influence the development and growth of the female accessory organs of reproduction. In fetal rabbits and mice which are experimentally deprived of the ovaries, the accessory reproductive organs show a continuation of differentiation (64, 113, 114). When the female reproductive tract of the fetal rat, minus ovaries, is explanted onto culture medium, the accessory organs develop as well as if the ovaries were present (112).

Destruction of the growing hypophysis in the mouse embryo by means of X-rays is followed by a reduced number of germ cells in the ovaries (113, 115). The present writer does not know if this observation points exclusively to the experimentally produced absence of a fetal hypophyseal gonadotropin or to such nonhormonal factor as a direct and injurious effect of the X-rays upon the female sex cells.

The development of the ovaries in fetal rats may be hastened experimentally by small doses of injected gonadotropin (128). This effect is not produced by large doses of chorionic gonadotropin (139).

D Overview

We still have unanswered questions about the role of the fetal gonads in sex differentiation. As an extension of Lillie's theory (77), but based also on additional observations, we have a published view that in males the testes bring about the differentiation of the male accessory organs and prevent the formation of female structures, and that in females the ovaries do not influence sex differentiation (65).

This view involves several interpretations, two of which may now be considered briefly. The first deals with the effects of an embryonic testis grafted in the ovarian region of a female rabbit fetus. It is thought that the grafted testis was a hormonal factor in the two abnormalities noted: persistence of the Wolffian duct on that side of the body, and disappearance of a segment of the homolateral Mullerian duct (66). This interpretation, apparently resting entirely on a single case, is not supported by the results of explanting the female reproductive tracts of fetal rats onto culture medium and of placing in the immediate vicinity of the Mullerian duct an explanted testis or a micropellet of testosterone; the Wolffian duct undergoes the usual retrogression and the Mullerian duct continues to develop (112).

The second interpretation is that the deprivation of a fetus of its testes causes a "feminization" of the fetus (65, 66, 113). As an example of such feminization, the presence of a "vagina" in a castrated male fetus is cited (66, 113). Other workers do not interpret their own results in this way (145, 146). When a male fetal rat is castrated before the prostatic buds appear and before the Mullerian ducts undergo all of their usual retrogression, the subsequent development includes the formation of fewer prostatic buds than normal and the formation of a prostatic utricle (uterus masculinus) which is sometimes larger than usual (distended with fluid). The ejaculatory ducts also are sometimes larger than normal (distended with fluid). Common denominators in the enlargement of both sets of structures, prostatic utricle and ejaculatory ducts, seem to be the absence of a patent outlet into the urethra.

(urogenital sinus) and the attendant accumulation of fluid in the luminated portion of each structure above (cranial to) the urethra. For these and other reasons, the present writer objects to any statement that a castrated male fetus is "completely feminine"; see Wells' discussion of a colleague's paper, on page 159 of that paper (66).

IV. ANATOMY OF THE REPRODUCTIVE ORGANS AFTER BIRTH

A. Ovary

The ovary may produce "good eggs and bad eggs." In the opossum (*Didelphis*) the formation of bad eggs seems to be due largely to an inherent lack of growth potential and not to a deficiency in the maternal environment (53).



FIG. 10. Section of an ovary from a kitten of 37 days. On the surface (top) among the cells of the germinal epithelium is a large ovogonium which seemingly has originated from this epithelium. The photograph is reproduced with the permission of Dr. Moore, now Surgeon and Director of the Roswell Park Memorial Institute, Buffalo, New York. (G. E. Moore, unpublished.)

It is generally believed that the ovary produces new ova after birth (Fig. 10); the writer thinks it would be difficult to prove whether such cells are the direct descendants of the original sex cells that are thought to originate from the entoderm of the hindgut. The question of whether ovocytes are formed in adult life, by the mitosis of cells in the germinal epithelium of the ovary, has been considered and answered in the negative, the view being that with the exception of two mammals, guinea

pig and armadillo, this process probably does not occur after puberty (155, 156)

The accumulating evidence that oocytes are not produced anew during adult life includes the following observations (a) the total number of oocytes in the rat ovary decreases with advancing age, from the 60th to the 365th day (84), (b) the number does not vary significantly during the estrous cycle in rats (85) or during the menstrual cycle in monkeys (41, 42), (c) in a rat ovary that shows compensatory hypertrophy after unilateral ovariectomy, the total number of oocytes is within the usual range for one normal ovary (83, 86), (d) when the germinal epithelium of the rat ovary is destroyed by painting the ovarian surface with tannic acid, normal and atretic follicles persist in this ovary as long as 469 days after the treatment (82), (e) in the hypophysectomized female rat, the number of oocytes becomes larger than that in normal animals of the same age, possibly because hypophysectomy decreases the rate of decline in number with an advance in age (62)

This question of neoformation of ova has been investigated in a large number of mammals by Burkl (15, 16). Burkl concludes that the germinal epithelium (ovarian peritoneum) is not necessarily the sole source of ovogonia during maturity. Thus, he believes that new ovogonia are formed after puberty.

The corpora lutea have been studied in the rhesus monkey (24) and in the rat (76). The corpus in the ovary of the monkey becomes fully organized by the 7th to 9th day after ovulation. Important steps in this process include the metamorphosis of the granulosa cells into lutein cells and the vascularization of the corpus. Signs of degeneration of a corpus begin to appear by the 13th day after ovulation. The degenerating corpus shows lipid vacuolation, nuclear pyknosis, cellular shrinkage, and cytolysis. The corpus luteum in the rat, on examination by electron microscopy, shows a subendothelial space which contains amorphous material of electron density equal to that within the lutein cell. This material is believed to cross directly the plasma membrane which in places is discontinuous.

The interstitial tissue of the ovary in the rat is first derived from granulosa cells, a phenomenon that ceases at about the 18th day after birth (27). Thereafter, especially after the first ovulation, the interstitial tissue arises by the transformation of cells of the theca interna. During this process the newly formed interstitial cells acquire droplets of lipid, and they soon begin to secrete estrogen, which is demonstrable in sections of the ovary (17).

The interstitial cells in the rabbit ovary have granules of lipid (22)

These granules, containing esterified cholesterol, are regarded as precursors of estrogen.

Comparative studies of the ovarian interstitial cells in carnivores (106), ungulates (107), and other mammals (108) bring out a wide range of patterns associated with sexual periodicity. The ovaries of certain wild mammals (wildcat and fox) have more interstitial tissue than those of domesticated forms (cat and dog).

Histochemical studies of the ovaries have been made in women (75), cattle (92), and rats (28). In rats, alkaline phosphatase and ascorbic acid are especially rich in the sites of formation of steroid hormones, namely, the theca interna of normal follicles, the granulosa of follicles becoming atretic, the corpora lutea, and the interstitial tissue.

The fine structure of the stroma of the human ovary includes a net of fibers in all stromal regions (30). A delicate layer of fibers surrounds the primary follicle.

The lymphatic drainage of the human ovary has been studied by injecting a dye (pontamine sky blue) and a spreading agent (hyaluronidase) into one ovary and noting the spread of the dye (33). The most common route is along the ovarian blood vessels. Alternate routes are: along the uterus and uterosacral ligaments to the hypogastric nodes, along the round ligament of the uterus to the external iliac nodes, and upward via the broad ligament and ureter.

Compensatory hypertrophy of the ovary after unilateral ovariectomy in the guinea pig is mediated by the anterior hypophysis (1). The hormonal mechanism seems to involve a neurohypophyseal reflex.

Ovarian tissue of the rat may be pretreated with glycerol and then frozen for as long as a year and finally transplanted successfully (105). Ovaries frozen and then grafted into spayed rats lead to cornified vaginal smears in about 15 days (29).

Biopsied human ovarian tissue has nodules of decidua-like tissue in a high percentage of cases (63). It is said that this condition can be distinguished from the pathological conditions of endometriosis and granuloma.

The recent literature on the human ovary includes records of gonadal dysgenesis (109) and of islands of adrenocortical tissue in the ovary (120). Such islands occur in prenatal and adult life.

B. Uterine Tubes

Each of the two uterine tubes in man seems to have a muscular sphincter in the infundibulum (125, 126). In fact, the musculature of the tube is made up of spiral bands, one of which is situated in the in-

fundibulum (57) Individual spiral bands may be followed into the fimbriae of the tubal ostium (57, 126, 127)

The so-called interstitial portion of the human uterine tube, also known as the isthmus uteri, does not seem to have a morphologically demonstrable sphincter (78) A functional sphincter may be demonstrated radiologically (9) The functioning of this sphincter is reported to be influenced by estradiol and progesterone

The epithelium of the tube in the rabbit consists of two types of cells, secretory and ciliated, it has been examined by electron microscopy (10). Only the secretory cells exhibit cyclic changes associated with the reproductive cycle The extracellular portion of a cilium is composed of 11 fibrils, 9 around a central pair Each of the 9 peripheral fibrils may be actually a pair of smaller elements

In the isthmus of the tube in the rat, alkaline phosphatase is found in the apices of the epithelial cells (28) This suggests the transfer of phosphorylated compounds across the cellular membrane at that site

Of interest from the standpoint of diabetes is the report of clear cells of Feyrter in the mucosa of the human uterine tube (94) Such cells can be demonstrated in the tube before birth, in maturity, during pregnancy, and in the menopause

Cyclic changes in the mucosa of the tube have been described and related to the estrous cycle in cattle (79) and sheep (48)

C Uterus

In these days when workers use electron microscopes and tokodynamometers (116) to study the uterus, it is well to remind ourselves of our scientific heritage One reminder is an account of the discovery of smooth muscle in the uterus (25)

The myometrium has been investigated in man (26, 88), rat (80, 88), and cow (111) In the human myometrium, irregularly scattered strands of muscle are found in the cervix They are embedded in a collagenous matrix The contractility of the cervix is, of course, negligible In the myometrium of a pregnant rat, the muscle fibers increase in length, but the diameter and density of individual myofilaments show no change, these features are observable with the electron microscope (88) The duplex type of uterus has a complex pattern of muscular bundles (80) In the bovine uterus, the circular layer of muscle has a spiral pattern (111)

The endometrium of primates and the changes associated with the menstrual cycle have been considered in great detail An early study deals with the uterine glands in man the glands having been isolated for study by maceration by the Schweigger Seidel technique (99) The

four phases of the endometrial cycle are indeed well-known in man (5) and monkey (6), thanks in part to the fact that the cyclic changes in the reproductive tract of the female monkey have been worked out in greater precision and detail than in any other primate (51).

Special structures in the endometrium of certain ungulates are the endometrial cotyledons or caruncles. In cattle these cotyledons assure attachment of the chorion of a developing embryo; the epithelium of a cotyledon disappears by the 25th day of pregnancy, and the chorion is attached to the cotyledons by the 36th day (35). Attachment to the cotyledons begins about 800 hours following ovulation (88a). The anatomy of the cotyledon in pregnancy is recorded (54). In the fallow deer, the cotyledons are arranged along the mesometrial aspect of the uterus (50). Systematic histochemical studies of the bovine endometrium have been made (93, 124).

Metrorrhagia, a normal process in the bovine endometrium, is not directly comparable to menstruation in primates. Such metrorrhagia is a postestrous phenomenon and is found in the cotyledons (48a) and the intercotyledonary areas (135). The bleeding may be furthered by heparin, an anticoagulant, which may be produced by tissue mast cells abundant in the endometrium (136).

The pregnant equine uterus develops endometrial cups which contain gonadotropin (23). This hormone seems to be secreted by the uterine glands in the area of the cups.

Several studies deal with eosinophilic granular cells of the human endometrium. These modified stromal cells (49) have in their cytoplasm few or several large granules, from 1 to 20 per cell (55). The granules are rich in tyrosine and tryptophan (56). It is believed that the granules are (or contain) heparin and that the cells bearing them are tissue mast cells (119).

The granules of the eosinophilic granular cells in the metrial gland of the gravid rat exhibit, by electron microscopy, meridional bands (151). Histochemically these granules contain lysine; hence they may be related to relaxin, which is a simple protein with lysine, but not arginine, among its amino acid constituents.

The cervix uteri in man, when studied by electron microscopy, is found to contain fibers of precollagen and a cement substance (7). It is possible that the elasticity of the precollagenous fibers and changes in the cement substance account in part for the stretchability of the cervix during parturition.

In a histochemical study of the human cervix, all phases of the reproductive cycle and also the menopause are considered (149). No special correlation of the several variations is recorded by the authors.

The bovine cervix uteri has at least four large endometrial rugae or bands (Figs 5, 6, and 8). The same number is depicted for *Dama*, the fallow deer (50).

The lymphatic drainage of the human uterus has been studied by two techniques: injecting India ink and an X-ray-contrast medium (72), and injecting a dye, direct sky blue (103). The lymphatic vessels from the lower segment of the uterus traverse more lymph nodes than those from the upper segment (72).

D Vagina

The epithelial cells of the human vagina are bound together by protoplasmic bridges. In sections of epithelium studied by electron microscopy, there are no clear cell boundaries (4). Cytoplasmic fibrillae run from cell to cell.

In the vagina of the rat, the content of alkaline phosphatase tends to show two peaks, during proestrus and during metestrus (37). The quantity of this enzyme is not otherwise related to the estrous cycle.

Other work on the vagina deals with the vaginal rugae in man (102), with the acidity of the vagina in mammals (73), and with the effects of locally applied vitamin A and estrogen upon the vagina of the spayed rat (67).

REFERENCES

- 1 Aron, M., and Aron, G., *Acta Physiol Latinoam* 3, 53 (1953)
- 2 Asdell, S. A., 'Patterns of Mammalian Reproduction' Comstock Publishing, Ithaca, New York, 1946
- 3 Baetsch, P., *J Endocrinol* 7 (Proc) 111 (1951)
- 4 Bahr, C. F., and Moberger, G., *Z Geburtshilfe u Gynakol* 146, 33 (1956)
- 5 Bartelmez, G. W., *Carnegie Inst Wash Contribs to Embryol* 24, 143 (1933)
- 6 Bartelmez, G. W. (with collaboration of G. W. Gomer and G. G. Hartman), *Carnegie Inst Wash Contribs to Embryol* 34, 99 (1951)
- 7 Berwind, T., *Arch Gynaekol* 184, 459 (1954)
- 8 Block, E., *Acta Anat* 17, 201 (1953)
- 9 Borell, U., and Fernstrom, I., *Acta Obstet Gynecol Scand* 32, 7 (1953)
- 10 Borell, U., Nilsson, O., Wersall, J., and Westman, J., *Acta Obstet Gynecol Scand* 35, 35 (1956)
- 11 Boyden, E. A., *Anat Record* 52, 325 (1933)
- 12 Brambell, F. W. R., in 'Marshall's Physiology of Reproduction' (A. S. Parkes, ed.), 3rd ed., Vol. 1, Chapter 5 Longmans, Green, New York, 1956
- 13 Bulmer, D., *J Anat* 90, 123 (1956)
- 14 Bulmer, D., *J Anat* 91, 490 (1957)
- 15 Burkl, W., *Z Zellforsch u mikroskop Anat* 41, 421 (1955)
- 16 Burkl, W., *Z Zellforsch u mikroskop Anat* 43, 345 (1955)
- 17 Burkl, W., and Kellner, G., *Z Zellforsch u mikroskop Anat* 40, 361 (1954)
- 18 Burns, R. K., *Carnegie Inst Wash Contribs to Embryol* 30, 63 (1942)
- 19 Burns, R. K., *Arch anat microscop morphol expil* 39, 467 (1950)

20. Burns, R. K., in "Analysis of Development" (B. H. Willier, P. A. Weiss, and V. Hamburger, eds.), p. 462. Saunders, Philadelphia, Pennsylvania, 1955.
- 20a. Burns, R. K., *Arch. anat. microscop. morph. exptl.* **45**, 173 (1956).
21. Carr, E. B., *J. Anat.* **87**, 423 (1953).
22. Claesson, L., *Acta Physiol. Scand.* **31**, Suppl. 113, 53 (1954).
23. Cole, H. H., Clegg, M. T., and Boda, J. M., *J. Animal Sci.* **9**, 678 (1950).
24. Corner, C. W. (with collaboration of C. G. Hartman and G. W. Bartelmez) *Carnegie Inst. Wash. Contribs. to Embryol.* **31**, 117 (1945).
25. Corner, C. W., *Acta Physiol. Latinoam.* **3**, 67 (1953).
26. Danforth, D. N., *Am. J. Obstet. Gynecol.* **68**, 1261 (1954).
27. Dawson, A. B., and McCabe, M., *J. Morphol.* **88**, 543 (1951).
28. Deane, H. W., *Am. J. Anat.* **91**, 363 (1952).
29. Deanesly, R., *J. Endocrinol.* **11**, 197 (1954).
30. Dietel, H., and Ferner, H., *Zentr. Gynäkol.* **73**, 949 (1951).
31. Eckstein, P., and Zuckerman, S., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), 3rd ed., Vol. 1, Chapter 2. Longmans, Green, New York, 1956.
32. Eckstein, P., and Zuckerman, S., see reference 31, Chapter 6.
33. Eichner, E., and Bove, E. R., *Obstet. and Gynecol.* **3**, 287 (1954).
34. Ellenberger, W., and Baum, H., eds., "Handbuch der vergleichenden mikroskopischen Anatomie der Haustiere," Vol. 2. Parey, Berlin, 1911.
35. Foley, R. C., and Reece, R. P., *Agr. Expt. Sta. Univ. Massachusetts Bull.* **46**, 62 (1953).
36. Forbes, T. R., *Carnegie Inst. Wash. Contribs. to Embryol.* **30**, 11 (1942).
37. Ford, D. H., *Anat. Record* **125**, 261 (1956).
38. Cillman, J., *Carnegie Inst. Wash. Contribs. to Embryol.* **32**, 81 (1948).
39. Glenister, T. W., *Nature* **177**, 1135 (1956).
40. Craham, M. A., *Anat. Record* **119**, 469 (1954).
41. Green, S. H., and Zuckerman, S., *J. Endocrinol.* **7**, 194 (1951).
42. Green, S. H., and Zuckerman, S., *J. Endocrinol.* **10**, 284 (1954).
43. Green, W. W., and Winters, L. M., *Agr. Expt. Sta. Univ. Minnesota Tech. Bull.* **169**, 1 (1945).
44. Creep, R. O., *Ann. Rev. Physiol.* **18**, 433 (1956).
45. Grünwald, P., *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **136**, 78 (1937).
46. Gruenwald, P., *Anat. Record* **81**, 1 (1941).
47. Gruenwald, P., *Am. J. Anat.* **70**, 359 (1942).
48. Hadek, R., *Anat. Record* **121**, 187 (1950).
- 48a. Hammond, J., "Reproduction in the Cow." Cambridge Univ. Press, London and New York, 1927.
49. Hamperl, H., *Klin. Wochschr.* **32**, 665 (1954).
50. Harrison, R. J., and Hyett, A. R., *J. Anat.* **88**, 338 (1954).
51. Hartman, C. G., *Carnegie Inst. Wash. Contribs. to Embryol.* **23**, 1 (1932).
52. Hartman, C. G., *Ann. Reo. Physiol.* **14**, 499 (1952).
53. Hartman, C. G., in "Mammalian Germ Cells" (G. E. W. Wolstenholme, ed.), p. 253. Little, Brown, Boston, Massachusetts, 1953.
54. Hatch, R. D., *Am. J. Vet. Research* **2**, 411 (1941).
55. Hellweg, G., *Arch. pathol. Anat. u. Physiol., Virchow's* **329**, 111 (1956).
56. Hellweg, G., and Sandritter, W., *Klin. Wochschr.* **34**, 1049 (1956).
57. Herrligkoffer, K. M., *Z. Geburtshilfe u. Gynäkol.* **138**, 63 (1953).

58. Heuser, C. H., and Streeter, G. L., *Carnegie Inst. Wash. Contribs. to Embryol.* 29, 17 (1941).
59. Hoffman, J., "Female Endocrinology." Saunders, Philadelphia, Pennsylvania, 1944.
60. Homma, H., *Arch. pathol. Anat. u. Physiol., Virchow's* 326, 679 (1956).
61. Hunter, R. H., *Carnegie Inst. Wash. Contribs. to Embryol.* 22, 91 (1930).
62. Ingram, D. L., *J. Endocrinol.* 9, 307 (1953).
63. Israel, S. L., Rubenstone, A., and Meranze, D. R., *Obstet. and Gynecol.* 3, 399 (1954).
64. Jost, A., *Arch. anat. microscop. morphol. exptl.* 36, 271 (1947).
65. Jost, A., *Arch. anat. microscop. morphol. exptl.* 39, 577 (1950).
66. Jost, A., in "Gestation" (C. A. Villee, ed.), p. 129. Josiah Macy, Jr. Foundation, New York, 1957.
67. Kahn, R. H., *Am. J. Anat.* 95, 309 (1954).
68. Kitchell, R. L., and Wells, L. J., *Anat. Record* 112, 561 (1952).
69. Klempman, S., *S. Africon Med. J.* 31, 722 (1957).
70. Koff, A. K., *Carnegie Inst. Wash. Contribs. to Embryol.* 24, 59 (1933).
71. Krohn, P. L., and Zuckerman, S., *Ann. Rev. Physiol.* 15, 429 (1953).
72. Kubik, L., and Várady, K., *Anat. Anz.* 104, 18 (1957).
73. Lang, W. R., *Obstet. Gynecol. Survey* 10, 546 (1955).
74. Latimer, H. B., *Anat. Record* 125, 731 (1956).
75. Leckie, F. H., *J. Obstet. Gynecol. Brit. Empire* 61, 772 (1954).
76. Lever, J. D., *Anat. Record* 124, 111 (1956).
77. Lillie, F. R., *J. Exptl. Zool.* 23, 371 (1917).
78. Lisa, J. R., Gioia, J. D., and Rubin, I. C., *Surg., Gynecol. Obstet.* 99, 159 (1954).
79. Lombard, L., Morgan, B. B., and McNutt, S. H., *J. Morphol.* 66, 1 (1950).
80. Ludwig, K. S., *Acta Anat.* 15, 23 (1952).
81. Makowski, E. L., Prem, K. A., and Kaiser, I. H., *Science* 123, 542 (1956).
82. Mandl, A. M., and Zuckerman, S., *J. Endocrinol.* 7, 103 (1951).
83. Mandl, A. M., and Zuckerman, S., *J. Endocrinol.* 7, 112 (1951).
84. Mandl, A. M., and Zuckerman, S., *J. Endocrinol.* 7, 190 (1951).
85. Mandl, A. M., and Zuckerman, S., *J. Endocrinol.* 8, 341 (1952).
86. Mandl, A. M., Zuckerman, S., and Patterson, H. D., *J. Endocrinol.* 8, 347 (1952).
67. Marberger, E., Bocciabelbe, R. A., and Nelson, W. O., *Proc. Soc. Exptl. Biol. Med.* 89, 466 (1955).
66. Mark, J. S., *Anat. Record* 125, 473 (1956).
- 86a. Melton, A. A., Berry, R. O., and Butler, O. D., *J. Animol Sci.* 10, 993 (1951).
89. Meyer, R., *Zentr. Gynäkol.* 61, 2846 (1937).
90. Moore, C. R., *J. Exptl. Zool.* 94, 415 (1943).
91. Moore, C. R., *Arch. anat. microscop. morphol. exptl.* 39, 484 (1950).
92. Moss, S., Wrenn, T. R., and Sykes, J. F., *Anat. Record* 120, 409 (1954).
93. Moss, S., Wrenn, T. R., and Sykes, J. F., *Endocrinology* 55, 261 (1954).
94. Müller, H. C., *Zentr. Gynäkol.* 74, 1162 (1952).
95. Nelson, W. O., *Ann. Rev. Physiol.* 17, 443 (1955).
96. Nelson, W. O., *Acto Endocrinol.* 23, 227 (1956).
97. Nelson, W. W., and Greene, R. R., *Surg. Gynecol. Obstet.* 97, 1 (1953).
98. Nichols, C. W., *Am. J. Vet. Resorch* 6, 135 (1944).
99. O'Leary, J. L., *Am. J. Anat.* 43, 289 (1929).

100. Ober, W. B., and Bernstein, J., *Pediatrics* 16, 445 (1955).
101. Oehler, I. E., *Acta Anat.* 12, 1 (1951).
102. Orsós, F., *Ann. Chir. et Gynaecol. Fenniae* 46, Suppl. 68, 1 (1957).
103. Pappalardo, G., *Arch. Ital. chir.* 82, 169 (1957).
104. Park, W. W., *J. Anat.* 91, 369 (1957).
105. Parkes, A. S., *Acta Physiol. Latinoam.* 3, 158 (1953).
106. Patzelt, V., *Z. mikroskop.-anat. Forsch.* 61, 309 (1955).
107. Patzelt, V., *Z. mikroskop.-anat. Forsch.* 62, 187 (1950).
108. Patzelt, V., *Ergeb. Anat. u. Entwicklungsgeschichte* 35, 99 (1956).
109. Plate, W. P., *Acta Endocrinol.* 26, 101 (1957).
110. Politzer, G., *Anat. Anz.* 102, 271 (1955).
111. Preuss, F., *Morphol. Jahrb.* 93, 193 (1952).
112. Price, D., in "Gestation" (G. A. Villee, ed.), p. 173. Josiah Macy, Jr. Foundation, New York, 1957.
113. Raynaud, A., *Arch. anat. microscop. morph. exptl.* 39, 518 (1950).
114. Raynaud, A., and Frilley, M., *Ann. endocrinol.* 8, 400 (1947).
115. Raynaud, A., and Frilley, M., *Compt. rend. soc. biol.* 143, 959 (1949).
116. Reynolds, S. R. M., "Physiology of the Uterus," 2nd ed. Hoeber, New York, 1949.
117. Roberts, S. J., "Veterinary Obstetrics and Genital Diseases." Edwards, Ann Arbor, Michigan, 1950.
118. Rosa, P., *Bull. fédération soc. gynécol. et obstét. langue franç.* 5, 341 (1953).
119. Rumbolz, W. L., and Greene, E. G., *Am. J. Obstet.* 73, 992 (1957).
120. Sauramo, H., *Acta Obstet. Gynecol. Scand.* 33, Suppl. 2, 1 (1954).
121. Sawyer, C. H., and Kritchlow, B. V., *Ann. Rev. Physiol.* 19, 467 (1957).
122. Scammon, R. E., *Proc. Soc. Exptl. Biol. Med.* 23, 687 (1926).
123. Sisson, S., and Grossman, J. D., "The Anatomy of the Domestic Animals," 3rd ed. Saunders, Philadelphia, Pennsylvania, 1938.
124. Skjerven, O., *Acta Endocrinol. Suppl.* 26, 1 (1956).
125. Stange, H. H., *Zentr. Gynäkol.* 74, 1176 (1952).
126. Stange, H. H., *Arch. Gynäkol.* 182, 77 (1952).
127. Stange, H. H., *Zentr. Gynäkol.* 75, 1401 (1953).
128. Stange, H. H., and Drescher, J., *Arch. Gynäkol.* 187, 693 (1956).
129. Streeter, G. L., *Carnegie Inst. Wash. Contribs. to Embryol.* 32, 135 (1948).
130. Torrey, T. W., *Am. J. Anat.* 76, 375 (1956).
131. Trautmann, A., and Fiebig, J., "Fundamentals of the Histology of Domestic Animals" (Translated from the 8th and 9th German eds., 1949, by R. E. Habel and E. L. Biberstein.) Cornell Univ. Press (Comstock), Ithaca, New York, 1957.
132. Turner, G. D., "General Endocrinology" 2nd ed. Saunders, Philadelphia, Pennsylvania, 1955.
133. Valdés-Dapena, M. A., "An Atlas of Fetal and Neonatal Histology." Lippincott, Philadelphia, Pennsylvania, 1957.
134. Vilas, E., *Z. Anat. Entwicklungsgeschichte* 93, 263 (1932).
135. Weber, A. F., Morgan, B. B., and McNutt, S. H., *Am. J. Anat.* 83, 309 (1948).
136. Weber, A. F., Morgan, B. B., and McNutt, S. H., *Cornell Veterinarian* 40, 34 (1950).
137. Wells, L. J., *Surgery* 14, 436 (1943).
138. Wells, L. J., *Anat. Record* 94, 530 (1946).
139. Wells, L. J., *Proc. Soc. Exptl. Biol. Med.* 62, 250 (1946).

- 140 Wells, L J, *Proc Soc Exptl Biol Med* 63, 417 (1946)
- 141 Wells, L J, *Anat Record* 97, 409 (1947)
- 142 Wells, L J, *Anat Record* 108, 309 (1950)
- 143 Wells, L J, *Arch anat microscop morphol exptl* 39, 499 (1950)
- 144 Wells, L J, *Carnegie Inst Wash Contrb to Embryol* 35, 109 (1954)
- 145 Wells, L J, in "Gestation" (G A Villee, ed), p 187 Josiah Macy, Jr Foundation, New York, 1957
- 146 Wells, L J, Carrough, M W, and Maxwell, E L, *Anat Record* 118, 109 (1954)
- 147 Wells, L J, and Fraick, R L, *Am J Anat* 89, 63 (1951)
- 148 Wells, L J, and van Wagenen, G, *Carnegie Inst Wash Contrb ta Embryol* 35, 95 (1954)
- 149 Wheeler, J D, and Drnziger, S, *Obstet and Gynecol* 5, 739 (1955)
- 150 Winters, L M, Green, W W, and Comstock, R E, *Agr Exp Sta Univ Minnesota Tech Bull* 161, 1 (1942)
- 151 Wislocki, G B, Weiss, L P, Burgos, M H, and Ellis, R A, *J Anat* 91, 130 (1957)
- 152 Witschi, E, *Carnegie Inst Wash Contrb to Embryol* 32, 67 (1948)
- 153 Witschi, E, *Anat Record* 128, 642 (1957), *Science* 126, 1288 (1957)
- 154 Zuckerman, S, *Arch anat microscop morphol exptl* 39, 609 (1950)
- 155 Zuckerman, S, *Recent Progress in Hormone Research* 6, 63 (1951)
- 156 Zuckerman, S, *Acta Physiol Latinoam* 3, 198 (1953)

CHAPTER 2

Anatomy of the Male Reproductive Organs

LOGAN M JULIAN AND WALTER S TYLER

	Page
I Introduction	29
II Development of the Male Reproductive Organs	30
III Postnatal Anatomy of the Reproductive Organs	31
A Testis and Associated Structures	31
1 Testis	31
2 Excretory Ducts	36
3 Scrotum and Testicular Coverings	38
4 Descent of the Testis	41
B Accessory Sex Glands	41
1 Regional Anatomy	41
2 Prostate	42
3 Seminal Vesicles	47
4 Bulbourethral (Cowpers) Glands	48
5 Glands of the Ampulla of the Ductus Deferens	48
C Copulatory Organ and Associated Structures	49
1 Penis	49
2 Prepuce	54
References	55

I INTRODUCTION

Precise knowledge of the normal morphology of the male genital organs is essential as a means of accessing potential or kinetic functional activity. In the domestic animals, the female genital tract has been much more intensively studied than that of the male. Although the male genital tract has received attention in standard books of gross anatomy of the domestic animals (11, 69, 73, 74, 87, 94) and in books of microscopic anatomy of domestic animals, laboratory animals, and man (18, 27, 35, 89), systematic studies at various ages are relatively scarce. Seldom has a group of males in the various species of the large domestic animals been reared solely for the purpose of necropsy at precise stages of development. Thus, most of the descriptions to follow are based upon presumably normal adult specimens, with little or no knowledge of preceding reproductive history.

Morphology has been widely used as an index of functional activity of the male genital tract. Assay procedures based on changes in morphology of the seminal vesicle, prostate, bulbourethral, and vas deferens have been carefully reviewed by Dorfman (24). The morphology and function of homologous structures, based on location in the adult and

origin in the embryo, are not consistent throughout the male genital organs of the domestic species. An example of morphological inconsistency is found in the seminal vesicles, which are true vesicles in man and solipeds, compact lobulated glands in ruminants and swine, and absent in carnivores. Species differences in function, as indicated by histochemical tests, were observed in the seminal vesicles of laboratory animals by Bern (9).

II. DEVELOPMENT OF THE MALE REPRODUCTIVE ORGANS

An "indifferent" stage of development of the genital system exists in the early embryo. In this period, development has not progressed to a point to indicate whether the embryo is to be a male or a female. The gonads are present, as are two sets of ducts, the Müllerian ducts (paramesonephric), which will predominate if the embryo is a female, and the Wolffian ducts (mesonephric), which will dominate if the embryo is a male. These features are more adequately discussed in Chapter 1. At this point, the development of the male system is to be reviewed with comment on residual portions of the female ductal system which persist to varying degrees in the adult male.

Some of the mesonephric tubules and portions of the mesonephric duct form the epididymis. The mesonephric duct develops into the vas deferens. The seminal vesicles develop as evaginations from the mesonephric duct, near or at its termination. An ejaculatory duct is formed from the terminal segment of the mesonephric duct; however, this is of little concern in the domestic species (as discussed below). Since the vas deferens develops from the Wolffian duct, it must be assumed that the glandular elements of the ampulla of the vas originate from tissues of the Wolffian duct. The prostate and bulbourethral glands develop from the epithelium of the urethra.

Remnants of the female duct system may be found in the male. The distal portions of the Müllerian ducts fuse during the "indifferent" stage of development in anticipation of the production of a portion of the vagina and (depending upon the species) the body of the uterus. Thus, in some specimens of certain species a relatively complete, although miniature, female tubular system may be found. In other species such vestiges are less frequent in occurrence and when found are much less complete. The distal remnants of the Müllerian ducts are termed the uterus masculinus or utriculus of the prostate. The latter term is more commonly employed in reference to man, and notes (as in other species) the proximity of the remnant to the prostate gland. The posterior end of the uterus masculinus may end blindly or it may enter

the urethra. Its structure may be quite simple or may be rather complete with a lining suggestive of the endometrium, the presence of uterine glands, and a distinct myometrium. The reactivity of the epithelium of the structure to endocrine products depends upon the embryonic origin of the tissues represented, differing if the remnant represents the portion of the vagina formed from the urogenital sinus or if it represents portions of the Müllerian ducts (Chapter 1). A well-developed uterus masculinus is frequently found in the stallion. This structure is small in the boar, dog, and cat. There is disagreement as to its frequency of occurrence in the bull. Conaway (16) discusses variations in structure and response of the uterus masculinus to endocrine products within and between species. A report by Zuckerman and Groome (96) deals with the structure of the uterus masculinus in the dog, its response to estrogens, and the application of its study to investigations of spontaneous endocrinopathies.

III. POSTNATAL ANATOMY OF THE REPRODUCTIVE ORGANS

A. *Testis and Associated Structures*

1. *Testis*

The testes have a dual function: production of sperm and the secretion of sex hormones. In adult domestic mammals the testes are located in the scrotum; however, they develop in the dorsal portion of the abdominal cavity. Details of the structure and location of the scrotum and of the descent of the testes are presented elsewhere in this chapter.

The testes of the domestic animals have an elongated ovoid form. Nomenclature of various portions of the testis is complicated by the diverse positions in which it is located in the domestic species. The borders of the testis in the domestic mammals are best described as the free border, which corresponds to the anterior border in man, and the attached or epididymal border, which corresponds to the posterior border in man. The dorsal or anterior extremity and the ventral or posterior extremity are rounded. The medial and lateral surfaces may be compressed.

The gland substance of the testis is enclosed by a dense connective tissue capsule, the tunica albuginea. The tunica is inverted into the substance of the testis along the attached border forming the mediastinum testis. The mediastinum traverses the long axis of the testis and tends to be more centrally situated in the testis of the domestic animals than it is in man (36). Portions of the duct system, the vessels and the nerves supplying the testis are located in the mediastinum. Sheets of connective tissue, the septula testis, radiate from the mediastinum to-

ward the surfaces of the testis where they attach to the tunica albuginea. The septula testis divide the parenchyma of the gland into cone-shaped lobules with apices at the mediastinum and bases at the surfaces of the testis. The lobules are the functional units.

In the testes of the bull and ram, the lobules are incompletely separated as the septa consist of strands rather than sheets of connective tissue (Fig. 1). The mediastinum is poorly developed in the stallion. Thick, interconnected septa which contain blood vessels and ducts are

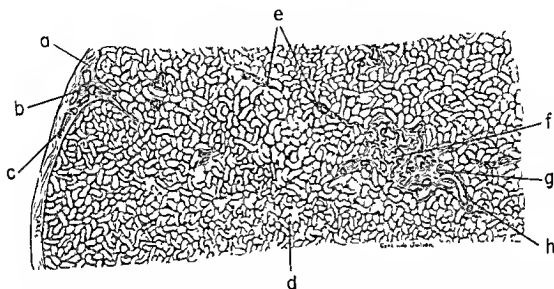


FIG. 1. Section of a testis from an 18-month-old Hereford bull. KEY: a, tunica albuginea; b, artery of the testis; c, vein of the testis; d, seminiferous tubule; e, strands of tissue forming the septula testis; f, rete testis in the mediastinum testis; g, artery in mediastinum testis; h, vein in mediastinum testis.

present (Fig. 2). These septa tend to converge on a disk-shaped area. Both the tunica albuginea and the septa of the stallion contain smooth muscle fibers (89).

According to conventional descriptions, each lobule contains two or three seminiferous tubules which have blind ends near the base of the lobule. Reconstruction techniques have indicated that arch-shaped tubules beginning and ending at tubuli recti near the apex are typical in the testes of dog, rabbit, and mouse (21, 43). Several arches may be linked together by Y- or T-shaped connections. The tubules pursue a very sinuous course in the peripheral portions of the lobule. These portions are referred to as the convoluted tubules. In the apices of the lobules the tubules follow a straight course and are referred to as tubuli recti. The total length of the seminiferous tubules have been estimated

in several of the domestic species. These investigations revealed that total tubule length is greater in the larger species, but that the smaller species have a greater length of tubules per unit body weight (4). They estimated the total tubule length in meters as follows: dog, 150; boar, 3000 to 6000; ram, 4000, and bull, 5000.

The walls of the seminiferous tubules consist of concentrically located connective tissue fibers, a basement membrane, and a multi-

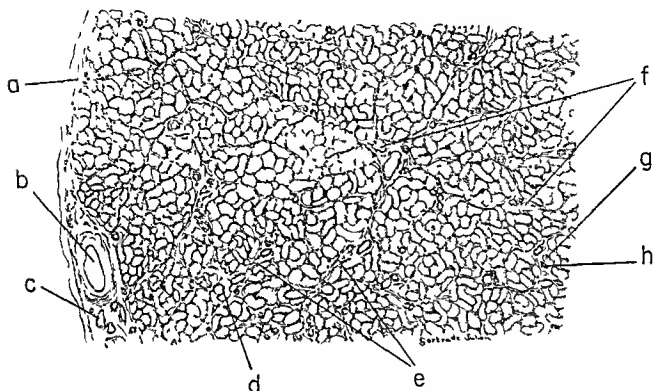


FIG. 2. Section of a testis from a 2-year-old stallion. KEY: a, tunica albuginea; b, artery of the testis; c, vein of the testis, d, seminiferous tubule, e, interconnected septa; f, collecting ducts homologous to rete testis; g, artery in a septum; h, vein in a septum.

layered epithelium. The epithelium consists of two fundamental types of cells; the spermatogenic cells and the sustentacular or Sertoli cells. The spermatogenic cells occupy concentric strata from the basement membrane to the lumen. These cells are directly concerned with the production of spermatozoa. The spermatogenic cells and the process of spermatogenesis are described in detail in Volume II, Chapter 1.

The Sertoli cells are attached to the basement membrane and extend centrally to the lumen. These cells are separated from each other by "pie-shaped" groups of spermatogenic cells. During certain phases of spermatogenesis the developing germ cells occupy indentations in the surface of the Sertoli cells. The cyclic nature of these cells was studied by Elftman (26). He reported accelerated maturation of the develop-

ing germ cells when they are surrounded by the Sertoli cells and emphasized their strategic location for the transfer of materials from the vascular bed outside the tubule to the embedded cells. Metabolic and histochemical techniques have been used to study the role of these and other cells of the testis (13, 57, 68, 70, 71, 72, 81, 92). Completeness of the Sertoli cell membrane of man has been demonstrated by electron microscope studies (29).

The stroma of the testicular lobule consists of a delicate connective tissue which contains vessels and nerves and the interstitial cells or cells of Leydig. The cells of Leydig occupy the triangular-shaped pockets between the seminiferous tubules. These cells tend to be oval-shaped with large spherical nuclei. The interstitial cells usually contain pigment granules which tend to increase with age. Pigment is especially pronounced during certain phases of the prenatal development of the horse testicle (15, 77). There is an abundance of evidence indicating that the cells of Leydig are the source of testicular androgens (42, 56, 81).

Two other hormones have been demonstrated in the testes of animals. Pregnenolone has been found in the testes of the boar (34). Estrogen has been isolated from male urine and testes (23, 25, 30, 95). Both the Sertoli and Leydig cells have been suggested as sites of the production of estrogen (10, 46, 56, 60). The main evidence suggesting the Sertoli cells as the source of testicular estrogens is the feminization in men and dogs with Sertoli cell tumors. The two main lines of evidence supporting the contention that the cells of Leydig are the source of this hormone are the estrogen excretion levels in men having only Leydig cells in the testis and the effects of chorionic gonadotropin in normal adult men. A definite answer does not appear to be available at the present time.

The prenatal and postnatal growth of the testes has been studied in most of the domestic species (1, 12, 15, 40, 54, 55, 77, 78, 80, 93). In a general way the growth patterns are similar, with the exception of the fetal horse. In the fetal horse the testes attain their maximum absolute size when the crown-rump measurement is from 45 to 65 cm. (15). At this stage the testes consist almost entirely of pigmented interstitial cells. Degeneration of these cells occurs later in intrauterine life; at parturition the testes are only approximately 1/10 of the maximum size attained earlier. Cole *et al.* (15) correlated these changes in the fetal horse testes with the hormonal content of the testes and of the maternal blood.

The principal artery to the testis is the internal spermatic or testicular

artery. This artery is a branch of the abdominal aorta which arises just posterior to the renal arteries. The blood supply to the testis develops while the testes are in the abdominal cavity and follows their descent to the scrotum. In domestic animals the artery is found in the vascular portion of the spermatic cord, in intimate contact with the pampiniform plexus of the corresponding vein (Fig. 3). This relationship is considered to be an important part of the testicular thermoregulatory mechanism by Harrison and Weiner (38). The techniques of dissection, arteriography, and microarteriography have been used by Harrison (36) to investigate the blood supply to the testes of a large number of

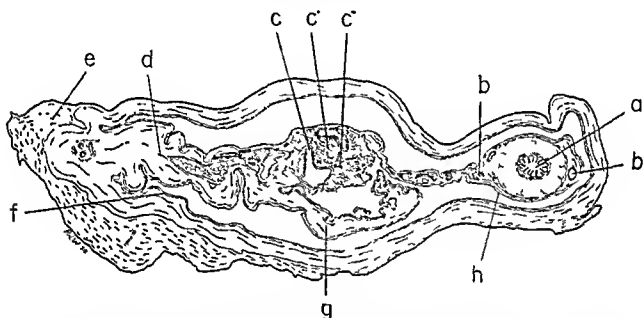


FIG. 3. Cross section of the spermatic cord and associated structures of a mature stallion. KEY: a, ductus deferens; b, arteries of the ductus deferens; c, vascular portion of the spermatic cord; c', spermatic vein; c'', spermatic artery; c''', spermatic nerve; d, internal cremaster muscle; e, external cremaster muscle; f, parietal vaginal tunic; g, fold of peritoneum; h, visceral vaginal tunic.

mammals, including most of the domestic species. The manner of venous drainage of the testes was found to be quite uniform, but differences were found in the manner of distribution of the testicular artery. In most species numerous veins from the medial and lateral surfaces coalesce on the dorsal (anterior) extremity of the attached border of the testis to form the pampiniform plexus which passes proximally in the vascular portion of the spermatic cord.

In the domestic animals the spermatic artery undergoes numerous convolutions in the spermatic cord. While on the attached border of the testis it supplies branches to all parts of the epididymis. On the testis the artery is found on the deep surface of the tunica albuginea. After reaching the ventral (posterior) extremity, the artery typically

divides into several convoluted branches which ascend the free (anterior) border. Smaller branches from these arteries supply the medial and lateral surfaces. Terminal arteries branch from these vessels, penetrate the substance of the testis and pass centrally toward the mediastinum.

Specific studies of the vessels after they enter the substance of the testis are available only for man and the bull (37, 52). The results of these studies are contrary to the older views of vascularization. In the two species investigated, the terminal arteries follow the septula testis centrally to the mediastinum, where they turn and again run peripherally. Numerous fine branches supplying the testicular parenchyma are formed as the vessels pass peripherally. Deferential arteries, indirect branches of the internal pudic arteries, were observed by Kirby (52) to anastomose with the spermatic artery in all of the bull testes examined. The positions of the deferential arteries of the stallion are depicted in Fig. 3. The external spermatic artery (cremasteric artery), usually an indirect branch of the external iliac, also anastomoses with the internal spermatic artery. These anastomoses are not of sufficient size to prevent atrophy of the testis when the internal spermatic artery is ligated (49).

The spermatic artery of the dog and stallion does not branch at the ventral (posterior) extremity, but a single artery ascends the free border, supplying branches to the medial and lateral surfaces. The testicular artery of the boar forms two main branches at the posterior extremity (36).

2. *Excretory Ducts*

A system of excretory ducts conducts the sperm from the site of formation in the seminiferous tubules to the termination of the urethra. All functions of the duct system are not completely understood, but it is known to function in the transportation, storage, maturation, and nutrition of sperm (67). Sperm leave the seminiferous tubules via the tubuli recti, emptying into the rete testis. The rete consists of a series of interconnected, thin-walled tubules located in the mediastinum testis (Fig. 1). The rete is lined with a low columnar or squamous epithelium. At the dorsal (anterior) extremity of the testis, where the mediastinum comes to the surface, several efferent ducts originate from the rete. The efferent ducts are straight as they leave the rete, but soon form spiral coils, the coni vasculosi or lobuli epididymidis. The apices of the coni vasculosi point toward the mediastinum. The efferent ducts have thin, smooth muscle walls most highly developed in the portions forming the coni vasculosi. The epithelium consists of alternating groups of tall and

low cells. Bleblike outgrowths may occur in the epithelium. The tall cells have cilia which beat toward the epididymis. The low cells contain more numerous secretory granules and may form intraepithelial glands.

The efferent ducts unite to form the ductus epididymidis. Grossly the epididymis is divided into three portions: a head located on the dorsal (anterior) extremity, a body extending along the attached border, and the tail situated on the ventral (posterior) extremity of the testis. The body and tail of the epididymis are bound to the testis by folds of the visceral processus vaginalis which form the anterior ligament of the epididymis. The scrotal ligament or posterior ligament of the epididymis attaches the epididymis to the scrotum. The lobuli epididymidis form a major portion of the head. The ductus epididymidis is highly coiled in the head and body. It is less tortuous in the tail where it gradually merges with the ductus deferens (vas deferens). The walls of the distal portions of the ductus epididymidis contain more smooth muscle than those of the proximal portions. The epithelium consists of pseudostratified, ciliated columnar cells with stereocilia and tends to be higher in the head and body than in the tail.

Experimental evidence indicates that the pressure of fluid secreted into the seminiferous tubules is responsible for the movement of sperm from the convoluted tubules to the epididymis (88, 90, 91). These experimental findings are in agreement with the microscopic structure of relatively thin walls of the ducts other than the epididymis and ductus deferens.

The ductus deferens ascends the inguinal canal in a special fold of the vaginal tunic (Fig. 3). In the abdominal cavity it proceeds dorsally and posteriorly, loops over the ureter to the dorsal portion of the neck of the bladder where the right and left ducts lay side by side. In the abdominal and pelvic cavities the ductus is located in the medial edge of the genital fold. The right and left genital folds fuse in the region of the bladder. This fold is the homolog of the broad ligament of the uterus in the female. The ductus continues posteriorly in the wall of the pelvic urethra. The ductus empties into the lumen of the urethra independent of the ducts of the accessory sex glands. A fusiform enlargement of the ductus deferens, the ampulla, is found in the terminal portion located dorsal to the neck of the bladder and pelvic urethra (Figs. 4 and 5).

The wall of the ductus deferens consists of a thin connective tissue adventitia, a very thick tunica muscularis, and a pseudostratified columnar epithelium which typically bears stereocilia. The epithelium is

lower than that of the epididymis. The increase in diameter at the ampulla is due to the addition of glandular elements very similar to the glands of the seminal vesicle (Fig. 4). Ampullary glands are not found in the ductus deferens of the cat and are poorly developed in the boar (51, 89).

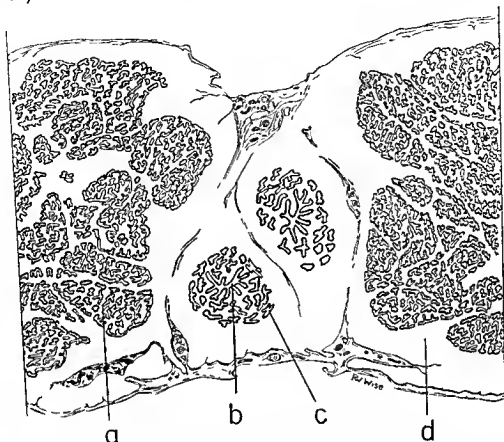


FIG. 4. Section of the seminal vesicles and ampullae of the ductus deferens of a mature Hereford bull. The section was taken immediately posterior to the neck of the bladder. KEY: a, glandular portion of the seminal vesicles; b, lumen of the ampulla; c, glands of the ampulla; d, smooth muscle fibers distributed in various planes.

The urethra is the common excretory duct for the genital and urinary systems of the male. The urethra is described with the penis and accessory sex glands.

3. Scrotum and Testicular Coverings

The scrotum is the pouch that contains the testes; its thermoregulatory function has been extensively studied (20, 75). The location of the scrotum in the domestic species varies from the inguinal region to the perineal region: in the stallion it is poorly developed and located

in the inguinal region; in the bull and ram it is well developed and is located in the anterior inguinal region; in the boar and tomcat it is found in the perineal region. The scrotum of the dog is situated approximately midway between the inguinal region and the anus. In domestic fowl, which lack a scrotum, the abdominal air sacs apparently assume some of the thermoregulatory functions of the scrotum (19).

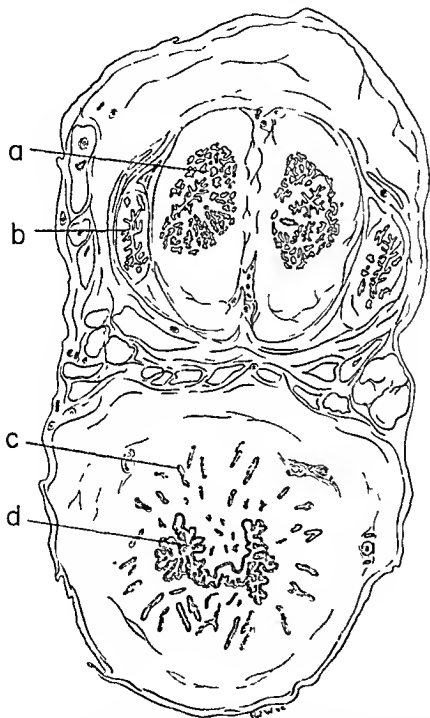


FIG. 5. Section of the pelvic urethra of a mature Hereford bull. This section was taken between the sections represented in Figs. 4 and 6. KEY: a, ampulla of the vas deferens; b, duct of seminal vesicle; c, corpus cavernosum urethrae; d, urethra.

The wall of the scrotum from the exterior inward is composed of the skin, tunica dartos, superficial scrotal fascia, deep scrotal fascia, external cremaster muscle, and the parietal processus vaginalis (39). These same layers may be found in other portions of the abdominal wall. The median line of the scrotum is indicated by the scrotal raphe. The serotal septum, a median division, is formed by the tunica dartos. The tunica dartos consists of smooth muscle fibers mixed with collagenous and elastic connective tissue fibers. The external cremaster muscle is striated and is derived mainly from the internal abdominal oblique muscle.

An evagination of the peritoneum, the processus vaginalis, lines each scrotal half. The parietal processus vaginalis, the tunica vaginalis communis, lines the scrotum and is continuous through the inguinal canal with the parietal peritoneum of the abdomen. At the posterior (dorsal) portion of the scrotum, the parietal layer is reflected over the epididymis, testis, and the structures of the spermatic cord forming the mesorchium and becoming the visceral or proper vaginal tunic. A small portion of the epididymis is not covered by the visceral vaginal tunic, but is connected to the scrotal wall by connective tissue which forms the scrotal ligament or posterior ligament of the epididymis. The scrotal ligament is the remnant of the gubernaculum. The cavity of the processus vaginalis is continuous with the peritoneal cavity of the abdomen through the vaginal ring.

The multiple layers of fascia in the wall of the scrotum allow a high degree of mobility of the testicle. The testes and scrotum may be elevated by either the tunica dartos muscle or the external cremaster muscle. Contraction of the cremaster also elevates the scrotum, as the testes and scrotum are connected by the scrotal ligament. Contraction of only the external cremaster does not result in maximal elevation of the testis (39). In thermal regulation of the testis, the tunica dartos muscle appears to be the more important of the two, as it reacts rapidly to any change of temperature. *In vitro* the tunica dartos muscle from adult rams starts to contract at 35 to 37°C. and reaches its maximum contraction at 20°C. (79). The development of the tunica dartos and its sensitivity to temperature changes is dependent upon testosterone. Mechanical distention of the scrotum may also be necessary for complete development (86).

The vessels and nerves supplying the scrotum appear to be dependent upon its location. If the scrotum is in the inguinal region, as it is in the stallion, bull, and ram, the vessels are branches of the external pudic artery and vein. The nerves to the scrotum of these animals are branches

of the second and third lumbar spinal nerves (3, 87). If the scrotum is located in the perineal region, as it is in the boar and tomtcat, it may receive part of its blood and nerve supply from the internal pudic vessels and nerves. This source appears logical as the scrotum develops in the perineal region as a homolog of the labia of the female. The labia are supplied by the internal pudic vessels and nerves. Lymphatic drainage from the scrotum passes to the superficial inguinal lymph node.

4. *Descent of the Testis*

The gonads develop retroperitoneally in the abdominal cavity medial to the developing mesonephros. The ligamentum testis develops in the genital ridge immediately posterior to the developing gonad. This ligament is continued across the body wall by the chorda gubernaculi which is in turn continued by the ligamentum scroti. These three structures unite to form the gubernaculum testis which extends from the posterior pole of the testis to the scrotal swellings. Differential growth results in a relative change in position of the testis with movement toward the pelvic cavity. A fold of peritoneum, the processus vaginalis, evaginates through a slitlike passage in the abdominal wall, the inguinal canal, and enters the scrotum. At this stage the testis is still in the abdominal cavity near the inguinal canal. The actual descent of the testis appears to be activated indirectly by the hypophysis and directly by the sex hormones (2). The role of the gubernaculum in the process of descent is still in dispute. The position of the testes changes from retroperitoneal in the abdominal cavity to retroperitoneal in the posterior portion of the scrotal cavity. The processus vaginalis then folds around the testis and associated structures to form the mesorchium and visceral layer of the vaginal tunic.

In the domestic animals, as in most mammals, the neck of the processus vaginalis remains patent and the cavity of the processus vaginalis communicates with the peritoneal cavity (59). This is unlike the situation in man where the neck of the processus vaginalis is obliterated (32).

B. *Accessory Sex Glands*

1. *Regional Anatomy*

The accessory sex organs of the male are situated behind the neck of the urinary bladder and are closely related to the pelvic portion of the urethra. The openings of the prostate and seminal vesicles are close to the neck of the urinary bladder. Bulbourethral (Cowper's) glands are situated more posteriorly, embedded in skeletal muscles associated with the root of the penis at the ischial arch.

The duct of the seminal vesicle is closely related to the terminal opening of the vas deferens. There may be a common opening for both the termination of the vas and the seminal vesicle. In such a situation, the term "ejaculatory duct" is used to designate the common terminal portion. The ejaculatory ducts are usually relatively short; they may pass through the substance of the prostate to gain the lumen of the urethra near the openings of the prostate gland. In common domestic animals the ducts open separately (87). Trautmann and Fiebiger (89) emphasize the presence of characteristic collecting sinuses in the lobular arrangement of all accessory genital glands of the male. These appear to be less prominent in the prostates of man and the dog in which species they are simply considered to be large collecting ducts.

The arterial supply to the accessory sex glands is derived from visceral branches of the internal iliac arteries (hypogastric arteries). The vessels directed to the accessory glands are frequently those which supply the urinary bladder. In the domestic animals, these vessels are portions of the umbilical arteries which are retained in the adult circulatory system. Because of the high incidence of carcinoma of the prostate in man, the venous drainage of the gland and of the pelvis in general has received considerable attention. In attempting to explain the paradoxical establishment of metastases of prostatic carcinoma, Batson developed the concept of a vertebral venous system (5, 6). The concept of a segment of the venous system extending from the cranial to the pelvic regions which does not recognize the influences of conventionally conceived vascular flow patterns has considerable significance in problems of pathogenesis. This concept has been extended greatly by the work and discussions of Herlihy (41).

2. Prostate

Typically, the prostate gland consists of two portions, a body situated behind the entrances of the vasa deferentia and seminal vesicles into the urethra, and a disseminated portion (*pars disseminata*) consisting of small glandular elements extending for some distance along the urethra and lying beneath the urethral muscle. The relative proportions of the two parts differ between species. The prostate of the bull and the boar consists of a small body and a relatively large disseminated portion (Fig. 6). In contrast, the gland of the stallion and of the dog consists of a large body and a small disseminated portion. The *pars disseminata* is always covered by the striated urethral muscle. The body of the prostate is exposed at the anterior margin of that muscle. The glandular elements surrounding the urethra are frequently more dense dorsally and laterally and lie peripherally to the corpus cavernosum urethrae.

In species in which the bodies of the prostates are relatively large, the gland is frequently described as being divided into lobes. The arrangement may be quite complicated, as in rodents in which four to six lobes are recognized (28, 33) or in man in which five lobes are described. The arrangement may be relatively simple, as in the case of the dog in which the gland is divided into two lateral halves, the hemispheres. The basis for such divisions has been either the gross appearance of the gland with its evident gross lobation, or (as in man)

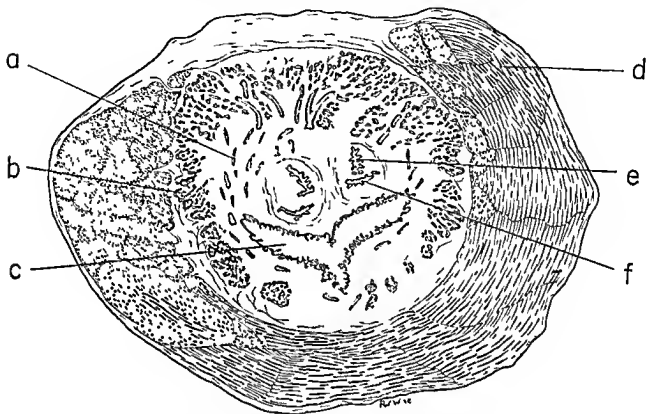


FIG. 6. Section of the pelvic urethra from a mature Hereford bull showing the pars disseminata of the prostate. KEY: a, corpus cavernosum urethrae; b, prostatic glands; c, lumen of the urethra; d, urethral muscle; e, terminal portion of the ductus deferens; f, duct of the seminal vesicle.

the divisions are based on studies of the development of the gland and the origin of each group of glandular elements (58). Concepts of lobation of the prostate glands of the domestic animals are based solely upon gross examination. Thus, the prostate of the horse does not completely surround the urethra, but consists of two lateral masses (lobes) connected by a dorsally placed "isthmus." Although complicated lobation is not described in the dog, functional lobation is suggested by reports of differing responsiveness to hormones of the glandular elements located ventrally to the urethra as compared to those situated dorsally (96). Thus, regional differences in response of the compact gland of the dog are similar to those described for the various individual

lobes of the prostate of rodents (53). In addition, regional differences in man are of interest because of the relative frequency of involvement of the various lobes in endocrinopathies and neoplasia (47).

In addition to the more complicated lobation described for the prostate of man, the glandular elements are grouped as mucosal, sub-mucosal, and main prostatic glands, indicating the site and relative

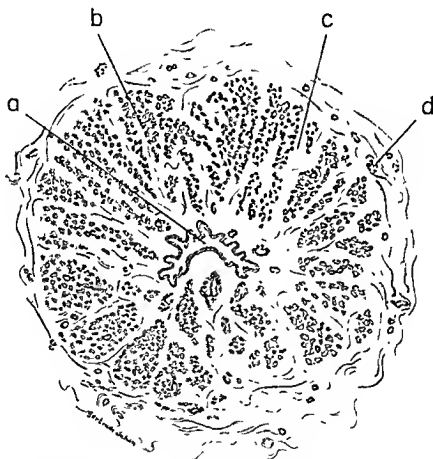


FIG. 7. Section of the prostate from a 24-hour-old Boxer puppy. Key: a, urethra; b, gland substance; c, septum; d, ganglionic cells.

extent of development. It is probable that the disseminated portions of the prostates of domestic animals correspond to the mucosal and sub-mucosal glands of the prostate of man. Trautmann and Fiebiger (89) consider the less well-developed, disseminated portions of the prostates of horses and carnivores to be similar to the glands of the urethral mucosa (glands of Littre). These points should receive further study as the determination of homologies of the various glandular elements of the domestic animals and man are complicated by such features as

the extent of urethra surrounded by the corpus cavernosum urethrae and comparative functional considerations of the various accessory glands, which are, in turn, reflected in great variation of structural features among the domestic species.

The glandular elements consist of a number of branched tubulo-alveolar glands opening individually into that portion of the urethra invested by the prostate gland proper. In immature animals the alveoli,

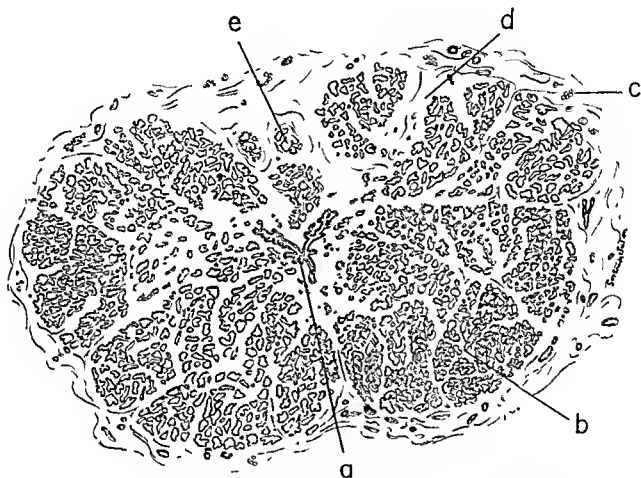


FIG. 8. Section of the prostate from an adult dog. KEY: a, urethra; b, gland substance; c, ganglionic cells; d, septum, e, ampulla of the ductus deferens.

which appear to comprise the majority of the gland in the mature animal, are small. The body of the gland is made up of the ductal system and stroma. Consequently, there is a low ratio of epithelium to fibrous and muscular tissue (Figs. 7 and 8). With enlargement of the alveoli at the onset of sexual maturity, due to androgenic stimulation, the epithelium becomes prominent. The alveoli are lined by a simple epithelium which has the characteristics of columnar, cuboidal, or squamous types, depending upon its functional stage. Moore (76) described the cyclic characteristics of the prostatic epithelium and the development and involution of the gland in man. The prostatic acini of man contain

concretions (*corpora amalacea*) composed of concentric layers of material surrounding a core of desquamated epithelial cells. Although described by many authors, these structures appear less frequently in the domestic mammals. The height of the lining epithelium as an indication of functional state of the prostate of the dog has been established by the volumetric functional studies of Huggins (47) and emphasized in the studies of Zuckerman (96). The epithelial cells of the prostate of sexually immature dogs are low cuboidal. The relation of height of epithelial cells and functional state does not hold for dogs with markedly enlarged glands exhibiting tall acinar epithelium (hyperplastic) (45). They secrete less prostatic fluid than normal dogs with glands one-tenth the size. This is another example of the basic biological problem, a prime challenge to functional anatomy, the relation of cell (or anatomical unit) number and structure to functional activity of an organ.

In certain details the microscopic characteristics of the prostate resemble those of the active mammary gland. However, it is distinguished by the presence of numerous smooth muscle fibers arranged throughout the stroma and to some extent circling the alveoli. The fibers are continuous with those of the capsule and thus they are more prominent in the larger stromal septa of the periphery of the gland. The smooth muscle is responsive to estrogenic stimulation. Because of its sensitivity to hormonal influence, the prostate has been of value in the bioassay of sex hormones (24).

There appear to be no studies of the arterial supply to the prostate gland of the domestic species comparable in detail to that of Flocks' study (31) of the gland of man. In the human the arteries directed to the prostate divide to form two groups of vessels, an inner or urethral group supplying the neck of the bladder and urethral portion of the gland, and a capsular group of arteries supplying the outer two-thirds of the gland. The latter group anastomoses, to a limited extent, with the inner group.

The draining veins of the prostate of the dog may be described as two groups, a superficial set ramifying over the surface of the gland and situated in the capsule of the gland. They extend centrally into the substance of the gland along the courses of the larger septa. A central group of veins, which appears to be extensions of the first group, join the venous spaces forming the *corpus cavernosum urethrae* (*corpus spongiosum*). It appears that most of the gland drains into the *corpus cavernosum urethrae* (51).

Numerous parasympathetic ganglionic cells may be seen in the

fibromuscular capsule and in the larger septa of the prostate (51) The presence of these is seldom emphasized in descriptions of the anatomy of the prostate nor in discussions of the autonomic nervous system Their demonstrable presence serves to emphasize the fact that secretion of the accessory sex glands is under the control of the parasympathetic system Preganglionic fibers reach these cells by way of the pelvic plexus, as do the postganglionic fibers of the sympathetic system which initiate discharge of the gland during the process of ejaculation

Because of the common use of the dog as an experimental animal by medical groups and because of special interest in the enlarged prostates of dogs past the age of five years, the gland of the dog has received a great deal of attention Information concerning the anatomy and pathology of the gland has developed from two directions Clinical studies have been conducted by Schlotthauer (82, 83, 84, 85) Anatomical and pathological studies directed toward the potential medical implications of the prostate of the dog are illustrated by the work of Zuckerman and co-workers (96, 97) and of Huggins (44) The comparative pathology of the prostate gland of man and the dog was reviewed in 1947 (50) and again in 1958 (8) It was found that the "benign hyperplasia" of aging dogs was not strictly comparable to the similarly named condition of man However, these studies have clearly illustrated the response of various tissue elements of the prostate (epithelium and smooth muscle) to sex hormones and these features have contributed to the establishment of suitable hypotheses concerning the underlying process of the condition in man

Whatever the implication in reference to pathology of man, the work of Zuckerman and Groome (96) contains the most comprehensive coverage of the microscopic and subgross anatomy of the prostate of the dog, and the works of Schlotthauer contain numerous valuable quantitative anatomical features Additional quantitative features have been reviewed recently by Berg (7)

3 *Seminal Vesicles*

The seminal vesicles are paired, lobated structures situated close to the neck of the bladder, intimately related to the openings of the vasa deferentia As stated above, the openings of the seminal vesicles and the vasa deferentia are separate in the domestic animals These accessory glands are not present in carnivores

In ruminants and swine the lobes of the seminal vesicles are made up of compact glandular elements with relatively small central dilations In contrast, the lobes of the glands of man and the horse contain

large dilatations, each of which is surrounded by glandular elements. The gross differences in the structure of the seminal vesicles of the domestic animals—size of lobes, extent of cavitation, number of ducts emptying from each lobe, and the organization of secretory ducts to glandular elements—are reflected in the distribution of muscular and connective tissue elements.

Mann and co-workers (48, 61, 62, 63, 65, 66) have studied functional activity by histochemical techniques. Their work has been confirmed by Cons (17) and represents the type of investigation which should be extended on a comparative basis. By these means, much of the difficulty in arriving at an interpretation of true homologies might be overcome and a greater appreciation of the function of the various accessory glands in different species might be obtained.

4. *Bulbourethral (Cowper's) Glands*

Cowper's glands are paired glands situated near the ischial arch. They are surrounded by, or embedded in, the urethral muscle or the thickened continuation of that muscle, the bulbocavernosus (Fig. 9). The glands are missing in the dog, but are present in the stallion, bull, ram, boar, and cat. The glands are lobulated, the lobules being separated by connective tissue and smooth muscle. In the case of the horse, the lobules are separated by strata of striated muscle fibers which are portions of the urethral muscle (Fig. 9). The epithelial elements are columnar cells varying in height with the functional state.

5. *Glands of the Ampulla of the Ductus Deferens*

As stated above, the accessory genital glands of the male conventionally consist of the prostate, seminal vesicle, and bulbourethral (Cowper's) glands. The problem of including the ampullae as accessory sex glands appears to be one of definition. Since the ampullae are portions of the ductus deferens they should not be considered separate organs on a morphological basis. However, there can be no doubt that the glands of the ampullae do contribute to the ejaculate. In the stallion, this contribution has been quantitatively determined and separated from the secretions of the seminal vesicles by Mann (64). This work is based upon the finding of ergothioneine in the secretions of the ampullary glands, but not in those of the seminal vesicle.

Ampullary glands are present in the terminal portions of the ductus deferens of the stallion, bull, ram, and dog. These glands are poorly developed or absent in the boar and are absent in cats (51, 89). In the opinion of the authors, the glands of the ampullae should be considered as accessory glandular elements.

C Copulatory Organ and Associated Structures

1 Penis

The penis is typically composed of three masses of erectile tissue, two corpora cavernosa peni and a corpus cavernosum urethrae. The former constitute the major masses of the base (root) and the body of the penis. The corpus cavernosum urethrae surrounds the urethra and

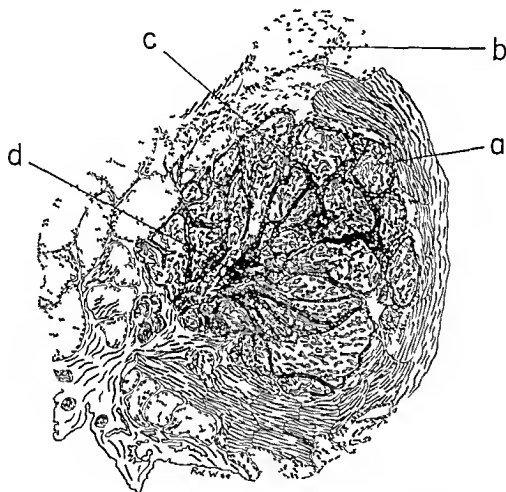


FIG 9 Section of a bulbourethral (Cowpers) gland from an 18 month old stallion. *key* a, glandular portion, b, urethral muscle, c, urethral muscle in gland septum, d, collecting sinus

contributes to the mass of the body of the organ. A fourth structure, the glans, completes the major masses of the organ. In some species the glans is mainly composed of erectile tissue, the vascular spaces of which are continuous with those of the corpus cavernosum urethrae.

The corpora cavernosa peni arise at the crura of the penis. Each crus is attached to the posterior and ventral margins of the corresponding ishium and is covered by an ischioavernosus muscle. The corpora converge to join the corpus spongiosum. The cross sectional shape of the penis of a given species is dependent upon the degree of fusion of

the two corpora cavernosa peni and the degree to which the corpus spongiosum is invested by the latter. In some, each major component is more or less defined in the superficial characteristics of the organ. In others, due to the forementioned fusion of the corpora cavernosa peni and their investment of the corpus spongiosum and the development of a heavy peripheral fibrous tunic, the penis is cylindrical in shape for the majority of its length (Fig. 10).

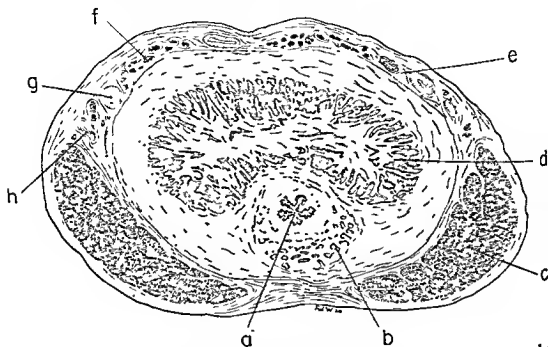


FIG. 10. Section of the penis from a mature bull. The section was taken at the point indicated in Fig. 12. Key: a, urethra; b, corpus cavernosum urethrae; c, retractor penis muscle; d, corpus cavernosum penis; e, tunica albuginea; f, nerve of the penis; g, artery of the penis; h, vein of the penis.

A fibrous tunic (tunica albuginea) surrounds each vascular body at its origin. These tunicae delineate the composing portions of the penis for varying distances from the ischial arch. The peripheral portions of the tunicae of the corpora cavernosa peni blend to form a continuous, heavy, supporting tunic for the entire penis. Along the line of apposition of the corpora peni, a septum is formed which extends forward toward the glans. It may be complete (recognizable for the entire length of the penis), as in the case of the dog (14), or it may become less distinct distally, as in the cases of the other domestic mammals and man. There are communications through the septum between the corpora cavernosa. Numerous trabeculae pass from the surrounding tunica albuginea into the mass of the corpus cavernosum, dividing the corpus into many spaces

and supporting the venous sinuses. The partitions are composed of fibroelastic tissue (ruminants and swine) and smooth muscle fibers (particularly evident in the stallion and carnivores) (89). In the domestic species, other than the cat, the vascular spaces of the corpus cavernosum are increased in size at the ischial arch to form a bilateral bulbous mass, the bulb of the urethra.

The degree to which each vascular cavernous body expands in the erect state is dependent upon such structural features as the thickness of the limiting tunicae and the composition of such tunicae and internal trabeculae. As examples, the corpora cavernosa of the penis of the bull and dog are heavy and are composed of a preponderance of collagenous connective tissue. In these species, the corpora cavernosa stiffen the penis during erection but do not greatly enlarge it. In contrast, the tunica albuginea and trabeculae of the corpora cavernosa of the horse and these elements of the glans penis of the dog are weaker and consist of goodly proportions of elastic fibers and smooth muscle.

The glans penis may be very small and contain little erectile tissue or it may be large, representing a major portion of the body of the penis. The glans is covered by stratified squamous epithelium which is continuous at the preputial frenum with the inner lining of the prepuce. Numerous specialized nerve endings are found on the glans and in the deeper tissues. In man, a number of varieties of nerve endings have been described. There appear to be no comprehensive comparative studies for the domestic animals. The glans penis of the cat presents a number of cornified projections which are directed distally. In the boar, the glans penis resembles a corkscrew in shape, exhibiting a shallow spiral groove.

An os penis is present in members of Marsupialia, Rodentia, Chiroptera, Carnivora, and some Primates. In the dog and cat, the bone is confined to the glans penis. In the dog, the bone is long and narrow. It bears a ventral groove through which the urethra passes. The anterior end is pointed and is composed of fibrocartilage. Illustrations of the bone are presented by Christensen (14) and Miller (69). The os penis of the cat is small (approximately 3 to 4 mm. in length) (Fig 11). Its proximal end is divided into three short projections, which reflect the bifid nature of the body of the penis. This structure is illustrated and described by Zietzelmann *et al.* (94).

The penis is supported from the ventral abdominal wall in most of the domestic animals; however, in the cat it is directed backward and downward from the ischial arch. The size, shape, relative length, and size differential in the flaccid and erect states vary greatly between

the species. The penes of ruminants and swine exhibit a sigmoid flexure. The flexure is sagittally placed; its lower curvature being maintained by the retractor penile muscles (Fig. 12). Urethral processes occur at the termination of the urethra in a number of the domestic animals. In the horse the process is short, somewhat centrally placed in the glans penis, and does not project much beyond the anterior extremity of the glans. It is divided from the glans by a sulcus. A dorsal extension of the sulcus forms a small blind pouch termed the urethral sinus. In

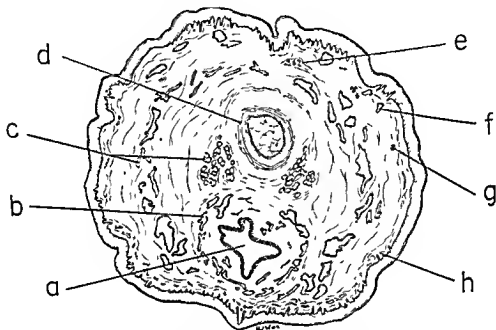


FIG. 11. Section of the penis from a 1-year-old tomcat. KEY: a, urethra; b, corpus cavernosum urethrae; c, corpus cavernosum penis; d, os penis; e, artery and accompanying nerves of the penis; f, vein of the penis; g, nerves of the penis; h, tunica albuginea.

the ram and goat, the urethral process extends beyond the glans as a twisted filament. The urethral process of the bull curves over the surface of the glans but does not extend beyond the tip of the structure (Fig. 12).

The penis is supplied by the deep and by the dorsal arteries of the penis. These usually arise as continuations of the internal pudic, a branch of the internal iliac. Some of the vessels may arise from the obturator artery in some species. The external pudic artery, which passes through the inguinal canal, usually complements the dorsal artery of the penis by anastomosis. At the ischial arch, another pair of important arteries arise from the internal pudic arteries. These are the

arteries of the bulb. They enter the bulb of the urethra and extend anteriorly in the corpus spongiosum. These comments present the general arrangement described for the origins of the vessels supplying the penis of domestic mammals, but minor modifications occur in each.

The veins of the penis are named for the arteries they accompany. Thus, penile venous drainage in the domestic mammals takes three courses: through the veins of the bulb of the urethra and through deep

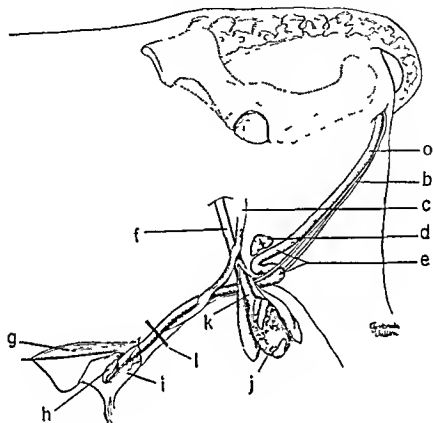


FIG. 12. Genital organs of the bull, lateral view. KEY: a, penis, b, retractor penis muscle; c, posterior preputial muscle; d, superficial inguinal lymph node; e, sigmoid flexure; f, external cremaster muscle; g, anterior preputial muscle; h, glans penis; i, preputial cavity; j, testis; k, spermatic cord; l, position of section portrayed in Fig. 10.

and superficial veins of the penis which drain by way of the internal pudic veins and the external pudic veins (through the inguinal canal). Of particular importance are the internal pudic veins whose compression by the ishiocavernosus muscle against the border of the ischial arch participates (in part) in the function of erection.

There are a number of features of the topographical and subgross angioarchitecture of the penis which have received considerable attention and which form the basis for current concepts of its erectile function. Coiled (helicine) arteries are described as primary branches of the arteries which supply the erectile portions of the penis. Special struc-

tural characteristics have been described for these arteries, including special modifications of their inner layers which are assumed to inhibit flow of blood when the penis is nonerect. The presence of some of these structural properties has been questioned (22). Special features have been described for the corresponding segments of the venous system; the thick walls of the deep veins have been emphasized particularly. In addition, strategically placed valves, as those described in the dorsal vein of the penis, appear to be of considerable importance in the maintenance of turgidity of the erectile tissues, as well as controlling the relative engorgement of the corpora cavernosa peni and the corpus cavernosum urethrae. Current concepts of the mechanism of erection include the synergism of two processes: (1) the expansion of the helicine arteries and the contraction of corresponding venules (under nervous control), permitting additional blood to enter the erectile tissues and slowing its exit, and (2) compression of the dorsal vein of the penis against the ischial arch, thus limiting total flow from the penis.

The lymphatic drainage of the penis passes to the superficial and deep inguinal nodes. The major nerve supply of the penis consists of the dorsal nerve of the penis. It arises from the sacral portion of the lumbosacral plexus by way of the pudic nerve. The nerve terminates at the glans penis and adjacent structures.

As stated above, the pelvic portion of the urethra is surrounded by an urethral muscle. This is continued at the ischial arch by the bulbocavernosus muscle which surrounds the extrapelvic portion of the urethra and extends a variable distance toward the glans penis. Another pair of skeletal muscles occur at the ischial arch. These are the ischio-cavernosus muscles which arise from the tuberos portions of the ischial arch, overlies the crura of the penis and converge to the midline to insert on the crura and body of the penis. When these muscles contract, pressure is exerted against the dorsal vessels of the penis, partially restricting the return flow of blood from the organ. This is one of the mechanisms responsible for erection of the penis.

In the domestic animals, two bands of smooth muscle arise from the ventral surfaces of the first few coccygeal vertebrae, pass downward to surround the terminal portion of the digestive tract and continue along the ventral surface of the penis to terminate in the anterior part of that organ near the frenum of the prepuce. These muscles, the retractor peni, function to return the penis into the sheath after protrusion.

2. Prepuce

The prepuce is an extension of the common integument over the glans penis in animals exhibiting a pendulous penis. In the domestic

quadrupeds, with the exception of the cat, the penis is held against the abdominal wall from its origin to its termination, usually just behind the umbilicus, by a fold of the common integument. Thus, the prepuce of man and of domestic animals is not strictly comparable. In the latter, the entire penis is loosely held by the prepuce. The external layer of the prepuce usually does not differ markedly from adjacent portions of the common integument. Frequently, the hair may be sparse and sebaceous glands may be more numerous. At the preputial orifice, the skin turns inward to form the parietal layer of the prepuce. The hair is lost and the sebaceous glands are modified to open directly onto the surface rather than by the hair follicles. The parietal layer passes backward a variable distance and then folds forward at the fornix to become continuous with the covering of the glans (visceral prepuce). Lymph nodules and specialized sebaceous glands are located on the parietal surface and at the fornix.

In the horse, a secondary fold occurs within the preputial cavity, constituting a "true prepuce." In the boar, a large diverticulum (the preputial diverticulum) occurs in the roof of the prepuce. Preputial muscles, derived from the cutaneous muscle, are described for the boar, dog, and bull, and are illustrated in Fig. 12. Although variable, four such muscles may occur, an anterior pair and a posterior pair. The former originate anterior and lateral to the umbilicus and insert at the preputial orifice. The posterior pair originate in the inguinal region and insert on the anterior part of the prepuce.

REFERENCES

- 1 Allen, B. M., *Am J Anat* 3, 89 (1904)
- 2 Arey, L. B., *Developmental Anatomy*, 6th ed. Saunders, Philadelphia, Pennsylvania, 1954
- 3 Arnold, J. P., and Kitchell, R. L., *Am J Vet Research* 18, 229 (1957)
- 4 Bascom, K. F., and Osterud, H. L., *Anat Record* 31, 159 (1925)
- 5 Batson, O. V., *Ann Surg* 112, 138 (1940)
- 6 Batson, O. V., *Ann Internal Med* 16, 39 (1942)
- 7 Berg, O. A., *Acta Endocrinol* 27, 129 (1958)
- 8 Berg, O. A., *Acta Endocrinol* 27, 140 (1958)
- 9 Bern, H. A., *Anat Record* 104, 361 (1949)
- 10 Berthrong, M. E., Coodwin, W. E., and Scott, W. W., *J Clin Endocrinol* 9, 579 (1949)
- 11 Bourdelle, E., *Anatomie Regionale des Animaux Domestiques*, Vol. 3, Baillyère, Paris, 1920
- 12 Carmon, J. L., and Green, W. W., *J Animal Sci* 11, 674 (1952)
- 13 Cavazos, L. F., and McIlampy, R. M., *Am J Anat* 95, 467 (1954)
- 14 Christensen, C. C., *Am J Anat* 95, 227 (1954)
- 15 Cole, H. H., Hart, C. H., Lyons, W. R., and Catchpole, H. R., *Anat Record* 56, 275 (1933)
- 16 Conaway, C. H., *J Mammalogy* 39, 97 (1958)

17. Cons, D. N., *J. Endocrinol.* 14, 304 (1957).
18. Cowdry, E. V., "Cowdry's Special Cytology," 2nd ed. Hoeber, New York, 1932.
19. Cowles, R. B., and Nordstrom, A., *Science* 104, 586 (1946).
20. Crew, F. A. E., *J. Anat.* 56, 93 (1921).
21. Curtis, C. M., *Am. J. Anat.* 24, 339 (1918).
22. Deysach, L. J., *Am. J. Anat.* 64, 111 (1939).
23. Dodds, E. C., Greenwood, A. W., and Callimore, E. J., *Lancet* 228, 683 (1930).
24. Dorfman, R. I., in "Hormone Assay" (C. W. Emmens, ed.), p. 291. Academic Press, New York, 1950.
25. Dorfman, R. I., Callagher, T. F., and Koch, F. C., *Endocrinology* 19, 33 (1935).
26. Elftman, H., *Anat. Record* 31, 381 (1950).
27. Ellenberger, W., "Handbuch der Vergleichenden Mikroskopischen Anatomie der Haustiere," Vol. 2. Paul Parey, Berlin, 1911.
28. Engle, E. T., *Anat. Record* 34, 75 (1926).
29. Fawcett, D. W., and Burgos, M. H., *Anat. Record* 124, 401 (1956).
30. Fee, A. R., Marrian, C. F., and Parkes, A. S., *J. Physiol. (London)* 67, 377 (1929).
31. Flocks, R. H., *J. Urol.* 37, 524 (1937).
32. Cray, H., "Anatomy of the Human Body" (C. M. Goss, ed.), 26th ed. Lea and Febiger, Philadelphia, Pennsylvania, 1954.
33. Creene, E. C., Anatomy of the rat. *Trans. Am. Phil. Soc. No. 27*, xl + 370 (1935).
34. Haines, W. J., Johnson, R. H., Goodwin, M. P., and Kuizenga, M. H., *J. Biol. Chem.* 174, 925 (1948).
35. Ham, A. W., "Histology," 3rd ed. Lippincott, Philadelphia, Pennsylvania, 1957.
36. Harrison, R. C., *Proc. Zool. Soc. London* 119, 325 (1949).
37. Harrison, R. C., and Barclay, A. E., *Brit. J. Urol.* 20, 57 (1948).
38. Harrison, R. C., and Weiner, J. S., *J. Physiol. (London)* 107, 48P (1948).
39. Hartig, F., *Zentr. Veterinärmed.* 2, 739 (1955).
40. Hauser, E. R., Dickerson, C. E., and Mayer, D. T., *Missouri Unto. Agr. Expt. Sta. Research Bull. No. 503* (1952).
41. Herlitz, W. F., *Med. J. Australia* 1, 661 (1947).
42. Hooker, C. W., *Am. J. Anat.* 74, 1 (1944).
43. Huber, C. C., and Curtis, C. M., *Anat. Record* 7, 207 (1913).
44. Huggins, C., *Physiol. Revs.* 25, 281 (1945).
45. Huggins, C., and Clark, P. J., *A.M.A. Arch. Pathol.* 30, 1178 (1940).
46. Huggins, C., and Moulter, P. V., *Cancer Research* 5, 510 (1945).
47. Huggins, C., and Stevens, R. E., *J. Urol.* 43, 705 (1940).
48. Humphrey, C. F., and Mann, T., *Nature* 161, 352 (1948).
49. Joranson, Y., Emmel, V. E., and Pilka, H. J., *Anat. Record* 41, 157 (1929).
50. Julian, L. M., *Cornell Vet.* 37, 241 (1947).
51. Julian, L. M., and Tyler, W. S., unpublished.
52. Kirby, A., *Brit. Vet. J.* 109, 464 (1953).
53. Korotkevsky, V., and Dennison, M., *J. Pathol. Bacteriol.* 41, 323 (1935).
54. Latimer, H. B., *Growth* 12, 123 (1949).
55. Latimer, H. B., *Growth* 19, 207 (1955).
56. Leach, R. B., Maddock, W. O., Tokuyama, I., Paulsen, C. A., and Nelson, W. O., *Recent Progr. in Hormone Research* 12, 377 (1956).
57. Long, M. F., and Engle, E. T., *Ann. N. Y. Acad. Sci.* 65, 619 (1952).

- 58 Lowsley, O S, *Am J Anat* **13**, 299 (1912)
- 59 Lunn, H F, *Proc Zool Soc London* **118**, 345 (1948)
- 60 Maddock, W O, Epstein, M, and Nelson, W O, *Ann N Y Acad Sci* **55**, 657 (1952)
- 61 Mann, T, *Biochem J* **40**, XXIX (1946)
- 62 Mann, T, *Biochem J* **40**, 481 (1946)
- 63 Mann, T, *Nature* **157**, 79 (1946)
- 64 Mann, T, *Recent Progr in Hormone Research* **12**, 353 (1956)
- 65 Mann, T, Davies, D V, and Humphrey, G F, *J Endocrinol* **6**, 75 (1949)
- 66 Mann, T, and Parsons, U, *Nature* **160**, 294 (1947)
- 67 Mason, K E, and Shaver, S L, *Ann N Y Acad Sci* **55**, 585 (1952)
- 68 Meyer, J, and Weinmann, J P, *Am J Anat* **101**, 461 (1957)
- 69 Miller, M E, 'Guide to the Dissection of the Dog,' 3rd ed Edwards Brothers, Ann Arbor, Michigan, 1952
- 70 Montaga, W, *Ann N Y Acad Sci* **55**, 629 (1952)
- 71 Montaga, W, and Hamilton, J B, *Anat Record* **109**, 635 (1951)
- 72 Montaga, W, and Hamilton, J B, *Anat Record* **112**, 237 (1952)
- 73 Montane, L, and Bourdelle, E, 'Anatomie Regionale des Animaux Domestiques,' Vol 1 Bailliere, Paris, 1913
- 74 Montane, L, and Bourdelle, E, 'Anatomie Regionale des Animaux Domestiques,' Vol 2 Bailliere, Paris, 1917
- 75 Moore, C R, and Quick, W J, *Am J Physiol* **68**, 70 (1924)
- 76 Moore, R A, *Am J Pathol* **12**, 599 (1936)
- 77 Nishikawa, Y, and Horie, T, *Bull Natl Inst Agr Sci (Japan) Ser C* **10**, 348 (1955)
- 78 Onuma, H, and Nishikawa, Y, *Bull Natl Inst Agr Sci (Japan) Ser C* **10**, 365 (1955)
- 79 Phillips, R W, and Andrews, F N, *Mass Agr Expt Sta Bull* **331** (1936)
- 80 Phillips, R W, and Zeller, J H, *Anat Record* **85**, 387 (1943)
- 81 Pollock, W F, *Anat Record* **84**, 23 (1942)
- 82 Schlotthauer, C F, *J Am Vet Med Assoc* **81**, 645 (1932)
- 83 Schlotthauer, C F, *J Am Vet Med Assoc* **90**, 176 (1937)
- 84 Schlotthauer, C F, and Bollman, J L, *J Urol* **47**, 702 (1942)
- 85 Schlotthauer, C F, and Bollman, J L, *Cornell Vet* **26**, 342 (1946)
- 86 Selye, H, *Anat Record* **85**, 377 (1943)
- 87 Sisson, S, and Crossman, J D, 'The Anatomy of the Domestic Animals,' 4th ed Saunders, Philadelphia, Pennsylvania, 1953
- 88 Toothill, M C, and Young W C, *Anat Record* **50**, 95 (1931)
- 89 Trautmann, A, and Fiebigel, J, 'Fundamentals of the Histology of Domestic Animals' (translated by R E Habel and E L Biberstein), Comstock, Ithaca, New York, 1957
- 90 van Wagenen, C, *Anat Record* **27**, 189 (1924)
- 91 van Wagenen, C, *Anat Record* **29**, 399 (1925)
- 92 Wislocki, G B, *Endocrinology* **44**, 167 (1949)
- 93 Yoa, T S, and Euton, O N, *Am J Anat* **95**, 401 (1954)
- 94 Zietzschmann, O, Ackerknecht, E, and Grün, H, 'Ellenberger-Baum Handbuch der Vergleichenden Anatomie der Haustiere,' 16th ed Springer, Berlin, 1943
- 95 Zondek, B, *Nature* **133**, 209 (1934)
- 96 Zuckerman, S, and Groome, J R, *J Pathol Bacteriol* **44**, 113 (1937)
- 97 Zuckerman, S, and McKeown, T, *J Pathol Bacteriol* **46**, 7 (1938)

CHAPTER 3

Role of Anterior Pituitary Gonadotropins in Reproductive Processes

MIRIAM E. SIMPSON

	<i>Page</i>
I Dependence of the Gonads upon the Anterior Pituitary	60
A Pituitary Ablation Effects on Reproductive System	60
B Substitution Therapy	66
II The Pituitary Gonadotropic Complex FSH and ICSH (LH)	67
A Synergism and Antagonism between Gonadotropins	72
III Prolactin as a Member of the Gonadotropic Complex	74
IV Chemical Fractionation of Pituitary Gonadotropins	75
V Species Specificity in Pituitary Gonadotropins	77
A Antihormones (Antibody Formation)	77
B Species Differences in Biological Response to Gonadotropins	78
VI Regulation of Production and Secretion of Pituitary Gonadotropins	78
A Action of Gonadal Steroids on the Pituitary	78
B Direct Action of Gonadal Steroids on the Gonads	79
C Interpretation of Cyclic Reproductive Phenomena in the Female	79
D Neural Control of Pituitary Gonadotropic Activity	80
VII Hormonal Factors Necessary for Ovulation	81
VIII Hormonal Factors Necessary for Establishment and Maintenance of Pregnancy	83
IX Hormonal Interrelations in Problems of Fertility	84
X Dietary Hormonal Interrelations in Reproduction	86
XI Gonadotropins in Body Fluids	88
A Gonadotropins in Body Fluids during Pregnancy	88
B Detection of Pregnancy by Bioassay of Serum and Urine	89
C Gonadotropins in Body Fluids of Nonpregnant Animals	90
XII Bioassay of Gonadotropins	90
A Assay of Pituitary FSH	91
B Assay of Pituitary ICSH	93
C Assay of Human Chorionic Gonadotropin (HCG)	94
D Assay of Equine Gonadotropin (PMS)	96
E Assay of Urinary Gonadotropins in Nonpregnant Human Beings	97
XIII Relation of Pituitary Gonadotropins to Those in Body Fluids	100
A Survival of Gonadotropins in the Circulation (Half Life)	101
B Ratio of Follicle Stimulating and Interstitial Cell Stimulating Potency in the Pituitary and Body Fluids	102
C Pituitary Stimulation by the Various Gonadotropins	105
Acknowledgments	106
References	106

DEPENDENCE OF THE GONADS UPON THE ANTERIOR PITUITARY

A Pituitary Ablation; Effects on Reproductive System

The dependence of the majority of the endocrine glands on pituitary stimulation was first experimentally established when the pituitary was successfully ablated. Body growth ceased at once, and the adrenal cortex, the thyroid, and the gonads all became atrophic. The functional control of these organs by the anterior lobe of the pituitary was further substantiated when successful replacement therapy was instituted. The atrophy of the gonads following removal of the pituitary, or more specifically of its anterior lobe, was shown first in the dog. The subsequent perfection of this operation in the rat (144, 146) made available an experimental animal which has proved valuable in investigations of pituitary control of the gonads. Many of our concepts regarding the function of the pituitary have consequently been based on the physiology of this animal. Some of the observations, at first considered to warrant generalization, have proved inadequate, and further clarification is coming gradually from extension of such studies to other mammalian species. The cessation of reproductive function following hypophysectomy has now been described in several species, including the rhesus monkey (147).

After hypophysectomy in females, ovulation ceases, corpora lutea no longer form, follicles do not ripen, and the ovary decreases in size. All follicles large enough for beginning antrum formation are overtaken by atresia. In the atretic follicle the granulosa cells and ova die and are removed by phagocytosis, leaving only the thecal envelope, which contributes to the interstitial tissue of the ovary. The interstitial cells themselves undergo regressive changes. Their nuclei become shrunken and pyknotic and the cytoplasm diminishes. The production of ovarian hormones, estrogen and progesterone, is curtailed. Due to inadequate support from ovarian hormones, the dependent accessory organs (oviducts, uterus, vagina, and mammary glands) cease their rhythmic functional changes, decrease in size, and return to a structure closely resembling the infantile condition (Figs. 1-8). If the female is immature at the time hypophysectomy is performed the reproductive tract remains in the infantile condition.

In the male the same sequence of changes in the reproductive tract follows hypophysectomy. The testes rapidly atrophy. Mature spermatozoa cease to be delivered to the epididymis; spermatids do not form and those already present do not continue their transformation into spermatozoa. Spermatozoa although formed in small numbers.

least for a period, do not continue their growth and differentiation but become detached from the seminiferous epithelium and are found free in the lumen in various stages of degeneration. As the number of cell layers lining the seminiferous tubules decreases, the tubules decrease in size. The interstitial or Leydig cells between the tubules undergo regressive changes similar to those seen in the interstitial cells of the ovary. The accessory organs, including the prostate, seminal vesicles,

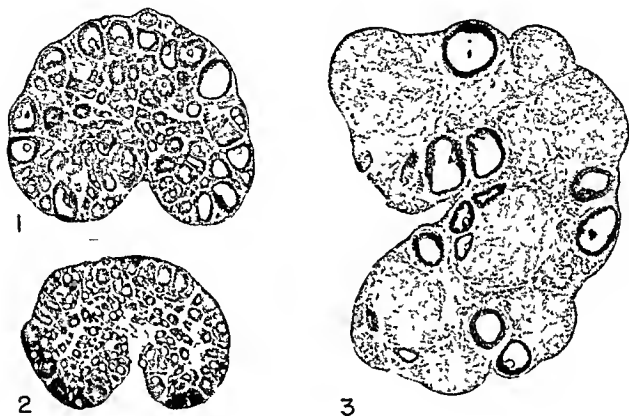


PLATE I Effect of hypophysectomy on the ovary (Hematoxylin and eosin stain, magnifications $\times 16$)

FIG 1. Ovary of normal immature rat, age 28 days.

FIG 2. Ovary of rat hypophysectomized at 27 days of age, 9 days postoperative showing regression in size of ovary and diameter of follicles.

FIG 3. Ovary of normal adult rat, age 71 days

lower's glands, and tubular pathways, deprived of the internal secretion from the Leydig cells, atrophy and in turn cease to secrete. If the operation is performed prior to maturity, testes and dependent reproductive organs fail to enlarge and differentiate and never become functional (Figs. 9-11).

Although full functional performance of the reproductive organs as thus been shown to be under control of the anterior pituitary, a certain minimum activity continues in the gonads after hypophysectomy. The primordial follicles in the ovary do not disappear, and a limited



PLATE II. Effect of hypophysectomy on the uterus. (Hematoxylin and eosin stain; magnifications: $\times 90$.)

FIG. 4. Uterus of normal immature female rat, age 28 days.

FIG. 5. Uterus of hypophysectomized immature (27-day-old) female rat, 10 days after the operation.

number even undertake some growth. The ovocytes enlarge and the granulosa cells multiply to form follicles with several granulosa layers in which an antrum may begin to form. The development does not con-

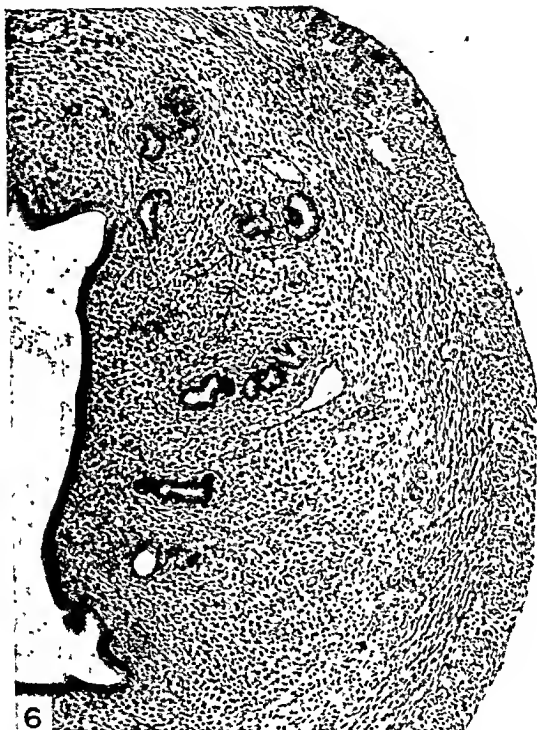


FIG. 6. Segment of uterus of normal adult female rat, age 71 days.

tinue further without pituitary support; atresia overtakes all such follicles at this or earlier stages of development, with death of both ovum and nurse cells. Such follicles do not reach sufficient development to produce estrogen in physiologically significant quantities. Even these

growing primary follicles diminish in number as the postoperative period lengthens.

In the male also, the gonads do not undergo complete degeneration. The tubules, although small, retain an orderly appearance (Figs. 9-11). In the immediate postoperative period, some accumulation of desquamated material is seen and multinucleate or fragmenting cells are present

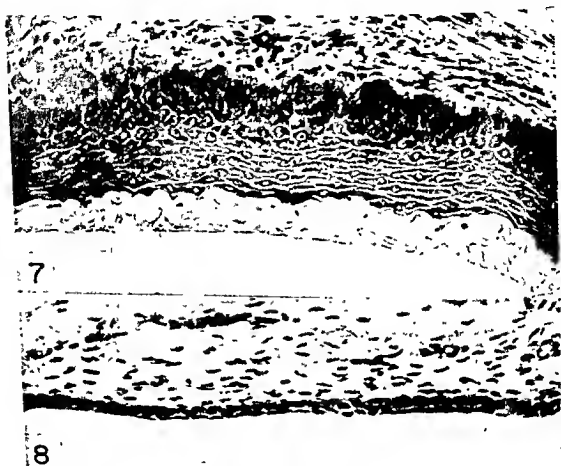


PLATE III. Effect of hypophysectomy on the vagina.

FIG. 7. Vagina of normal adult female rat, age 71 days.

FIG. 8. Vagina of hypophysectomized 27-day-old rat, 10 days after the operation. Note thinness of all tissue layers and especially flatness of the surface epithelium.

in the lumen. Eventually the lumen of the tubules becomes free of debris, and as the tubule shrinks the potential lumen also becomes smaller. The tubules are finally lined only by spermatogonia and Sertoli cells. Both cell types remain clearly distinguishable by their specific morphology and characteristic position. A few spermatocytes remain, but their number diminishes progressively with longer postoperative periods. The cytoplasm of the Sertoli cells, in which spermatids no



PLATE IV. Effect of hypophysectomy on the testis. (Hematoxylin and eosin stain; magnifications: $\times 95$.)

FIG. 9. Testis of rat hypophysectomized at 41 days of age, 15 days postoperative. Note regression from the condition shown in Fig. 10. Tubules contain only Sertoli cells and spermatocytes, many of which have desquamated.

FIG. 10. Testis of normal rat 41 days of age. Tubules contain spermatids.

FIG. 11. Testis of normal rat 56 days of age. During the 15-day period since the age shown in Fig. 10, spermatogenesis has progressed to the formation of mature spermatozoa.

longer become embedded, is clearly visible as filamentous strands, frequently constituting the only contents of the lumen of the tubule. Mitoses in spermatogonia, although uncommon, can be found for some time. Leydig cells are small but distinguishable from the stromal connective tissue cells in the intertubular spaces. The condensation of nuclear chromatin of the Leydig cells, which accompanies reduction in nuclear size, results in a peculiar pattern similar to that of the atrophic or "deficient" interstitial cells in the ovary.

This pattern of regression of the reproductive system, which is characteristic after surgical removal of the anterior lobe (or its complete destruction by any agent, such as radiation) has been described as it pertains to the rat. The sequence of changes is, however, typical of those which ensue in most species investigated. One peculiarity of the rat is the persistence of corpora lutea when the hypophysectomy is performed after sexual maturity. Although such corpora lutea are not functional, that is, do not produce progesterone, they retain their typical morphology indefinitely, whereas the life span of corpora lutea in the normal cyclic rat is only about 16 days.

B. Substitution Therapy

Reinstatement of the reproductive tract was first accomplished, at least with sufficient success to support the concept of the dependence of the gonads on the pituitary, by reimplantation of fresh pituitary tissue and by administration of saline suspensions of anterior pituitary (144, 146). Further confirmation of the dependence of the gonads on the pituitary was afforded by induction of precocious puberty. This was achieved almost simultaneously and independently by Smith (145) and by Zondek and Aschheim (176). After injection of pituitary substances into infantile rats and mice, puberty followed within 3 or 4 days.

There were some notable limitations, however, in the success achieved by pituitary therapy. In the hypophysectomized male rat, growth of the testis and resumption of spermatogenesis resulted, with reestablishment of accessory organs and even fertility (146). In the normal immature male, pituitary extracts had only a limited effect. No success was achieved in induction of precocious puberty. Though the Leydig cells responded, the development of the seminiferous epithelium was not accelerated. This failure in the immature male remains one of the unsolved enigmas, not only for the rodent, but for all species investigated. In the normal immature female, injection of pituitary extracts frequently resulted in ovulation, but this was not the case in the hypophysectomized female, in which follicular development was more

commonly followed by trapping of ova within luteinized bodies than by ovulation and true corpus luteum formation.

Persistent efforts have been made to isolate the substance or substances present in the anterior pituitary which are responsible for gonadal stimulation, and to determine whether these factors are distinct from those mediating other physiological actions of the pituitary. The growth-promoting potency of the pituitary was the first to be distinguished as separate from its gonadotropic action; adrenocorticotrophic and thyrotropic activities were later acknowledged to be due to separate substances. It is still a question whether all phases of stimulation and transformation induced in the gonads by pituitary extracts are due to a single gonadotropic substance or to multiple factors. It appears more probable that different phases of ovarian growth and activity, including follicular growth, corpus luteum formation, and repair of interstitial tissue, and production of the ovarian secretions, estrogen and progesterone, are under the combined hormonal control of several pituitary factors. In the male as well, the hormonal control of gametogenic and endocrine functions of the testis has not been entirely elucidated.

II. THE PITUITARY GONADOTROPIC COMPLEX: FSH AND ICSH (LH)

The gonadotropically active substances in anterior pituitary extracts have been found to be associated with proteins, and fractionation procedures adaptable to the separation of proteins have been applied in efforts to separate the gonadotropins from other biologically active or inert contaminants. Certain fractions, when tested in immature female rats, were found to cause growth of follicles; such follicles, although sometimes attaining full preovulatory size, did not progress to the formation of corpora lutea. The fraction remaining after removal of the follicle-stimulating component, although showing no gonadotropic activity when given alone to normal immature rats, resulted in corpus luteum formation when recombined with the first fraction. From such evidence it was concluded that these two phenomena, follicle growth and corpus luteum formation, are under separate hormonal control. The active principle in the first fraction was called the follicle-stimulating hormone (FSH); that in the second fraction, the luteinizing hormone (LH) (62). In hypophysectomized immature rats the first fraction stimulated follicular growth but did not lead to luteinization. The second fraction, added to the first, resulted in luteinization of the follicles. Given alone to hypophysectomized rats the second fraction had as its only gonadotropic action the repair of the deficient interstitial cells of the ovary. For this reason this fraction has also been designated

the interstitial cell-stimulating hormone (57) (Figs. 12-14). As this fraction has been found to have a similar action on the testis in the hypophysectomized male, namely, the repair of the deficient interstitial or Leydig cells, the designation interstitial cell-stimulating hormone (ICSH) may be preferable.

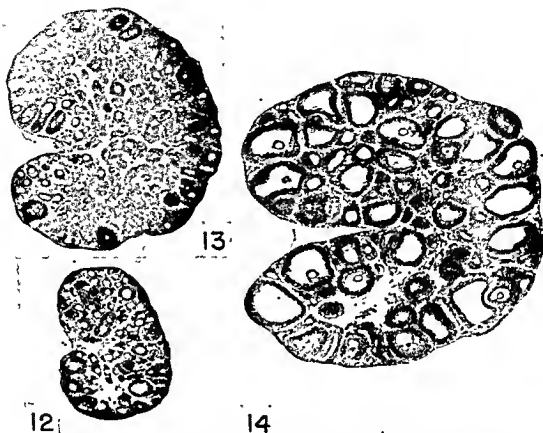


PLATE V. Ovaries of rats hypophysectomized at 26-28 days of age, 10-12 days after the operation. (Hematoxylin and eosin stain; magnification: $\times 19$.)

FIG. 12. Untreated control.

FIG. 13. ICSH treatment for 3 days. Note that interstitial tissue repair is the only effect of this pituitary fraction.

FIG. 14. FSH treatment for 3 days. Note development of many follicles to medium and large size, in the presence of deficient interstitial tissue.

Although pituitary fractions exert well-defined gonadotropic actions in the female in support of growth of germ cells, the relation which the two gonadotropins, FSH and ICSH, bear to maintenance and stimulation of the seminiferous epithelium of the testis is not as well clarified. It seemed probable at first that the follicle-stimulating fraction, which is gametokinetic in the female, leading to enlargement of the ovum and

multiplication of its sister granulosa cells, would have a similar action in the male. Thus it was anticipated that FSH would induce growth and differentiation of the male germ cells and perhaps their nurse cells, the Sertoli cells. The corollary that the Leydig cells, known to regress after hypophysectomy in a manner similar to the interstitial cells of the ovary, would respond like ovarian interstitial cells to the second fraction, ICSH, did prove correct.

The hypothesis of a gametokinetic action of FSH in the male, although still widely accepted as established, is on less secure grounds, and ICSH may prove to be the most important gonadotropic factor both for gametogenic and endocrine functions of the testis. The observation that the male sex hormone itself can maintain and even stimulate spermatogenesis (167) focused attention on the importance in the male of the second pituitary gonadotropic fraction, ICSH, because of its Leydig cell-stimulating and consequent androgenic activity. It appears possible, even probable at present, that ICSH is the gonadotropic pituitary fraction necessary in the male, both for spermatogenesis and for androgen secretion (Figs. 15 and 16) (139, 141). Further experimentation will be required to explain why spermatogenesis cannot be maintained for long periods by ICSH or by testosterone, and why neither readily reinstates spermatogenesis if a period of regression is allowed between hypophysectomy and replacement therapy. The importance of FSH in the male at some stage of tubular growth and differentiation, or in synergic action with ICSH, may still be established (142).

Table I shows the relative potencies of the various pituitary gonadotropins and testosterone in the hypophysectomized male rat, both their effect on Leydig tissue as reflected in growth of accessories, and their action on the seminiferous tubules as shown by the increase in testicular size and production of sperm. It will be noted that the pituitary ICSH (and nonpituitary interstitial cell stimulators as well, namely, human chorionic and equine gonadotropins) stimulated the seminiferous tubules at doses even lower than those which induced growth of the male accessory organs. It is assumed that testosterone produced by the stimulated Leydig cells was in all cases the active factor affecting the seminiferous epithelium. The action of injected testosterone propionate was manifested by testicular tubular development and accessory organ enlargement at approximately the same dose level. Purified pituitary FSH, on the other hand, had no effect on the accessory organs or on the tubules until such high doses were given that they were beginning to show that contaminating ICSH was present.

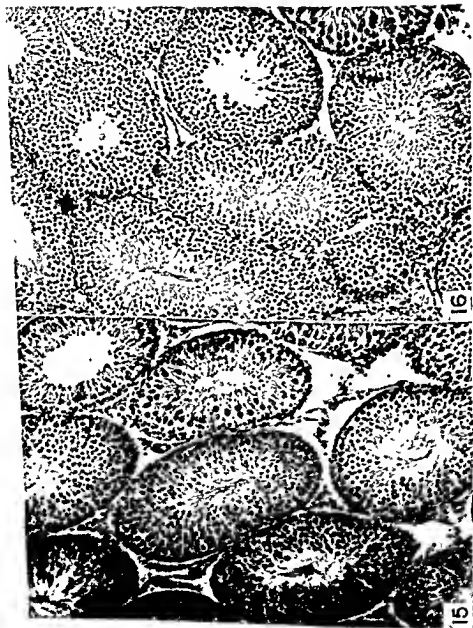


PLATE VI. Testes of rats hypophysectomized at 40 days of age, 15 days postoperative. (Hematoxylin and eosin stain; magnification: $\times 112$.)

FIG. 15. Treated with ICSH, 1 mg. daily, intraperitoneally, beginning the day of operation (13 injections in 15 days). Development has progressed to spermatozoan formation, as in the normal rat during this critical period (cf. Fig. 11).

FIG. 16. Treated with testosterone propionate, 1 mg. daily, subcutaneously in oil, beginning the day of operation (17 injections in 15 days).

TABLE I

COMPARISON OF THE SPERMATOCENIC AND ANDROGENIC PROPERTIES OF VARIOUS GONADOTROPINS WITH THOSE OF TESTOSTERONE PROPIONATE IN HYPOPHYSECTOMIZED 40-DAY OLD MALE RATS (139, 141, 142)

Material injected from 40 to 55 days of age	Daily dose (mg)	No of rats	Testes (mg)	Seminal vesicles (mg)	Prostate (total) (mg)	Spermatozoa in epididymus (%)
Testosterone	2.5	6	1737	1319	719	100
propionate	0.50	6	1604	1110	596	100
subcutaneous	0.25	16	692	675	399	40
	0.10	6	897	285	240	50
	0.050	5	638	80	126	0
	0.025	5	528	23	72	0
Human chorionic gonadotropin, subcutaneous	0.500	5	1710	998	586	100
	0.050 ^a	5	1716	217	211	100
	0.025	10	1705	141	117	100
	0.010	10	1429	79	65	100
	0.005	14	1070	18	47	70
	0.0025	17	915	18	52	40
	0.0010	5	515	15	44	0
Pituitary interstitial cell stimulating hormone, IP	1.0	5	1118	74	193	60
	0.050	5	1472	65	151	100
	0.010 ^b	8	1065	32	92	75
	0.0025	5	743	19	59	20
Pituitary follicle stimulating hormone subcutaneous	1.00	4	1702	18	64	100
	0.50	5	1188	16	48	80
	0.25	10	773	19	48	30
	0.050 ^b	12	643	16	40	0
Pregnant mare serum gonadotropin IP	0.035	5	2523	1187	542	100
	0.007 ^a	4	2080	206	245	100
	0.005	5	1712	97	141	100
	0.002	5	1572	27	76	100
	0.0005	4	539	16	37	0
Hypophysectomized 40 days 15 days post-operative		242	453	18	48	0
Normal 40 days		246	1354	42	129	0
Normal 55 days		52	2355	255	305	100

^a Minimal effective dose (MED) as assayed in normal immature female rats total dose in 4 days

^b MED as assayed in hypophysectomized immature female rats total dose in 3 days

The testis also is capable of producing estrogenic substances in great quantity in some animals, such as the stallion. The tissue of origin of this internal secretion of the testis has been attributed by some to the interstitial cells, and by others to Leydig cells as well, both concepts being based on the endocrine activity of certain testicular tumors (27, 87).

As FSH has been further purified, and further freed from contaminating ICSH, larger multiples of the minimal effective dose have been required to produce the estrous vagina and uterus. It could even have been anticipated that some ICSH would be required for full growth and secretory function of the follicle, inasmuch as the cells of the theca interna, forming the nutritive envelope of the follicle, have the same origin as the interstitial tissue and are likewise under the control of ICSH. No preparation of FSH has been freed entirely from ICSH; the more highly purified preparations usually contain between 1 part in 10 and 1 part in 40 of ICSH. It is therefore impossible at present to determine with certainty whether a small amount of ICSH is requisite for estrogen production, or whether there is a certain optimum proportion of FSH and ICSH which is needed. Students of the subject are not even agreed on the tissue of origin of the estrogen, some being inclined to the view that it is the thecal component of the follicle, the theca interna, and its derivative interstitial tissue which secrete estrogen, rather than the granulosa wall of the follicle.

A. Synergism and Antagonism between Gonadotropins

The biological activity of gonadotropic fractions could be interpreted more readily if it were not for the complex interrelations which exist between these hormones. For example, doses of ICSH too low to cause corpus luteum formation when given simultaneously with FSH nevertheless augment its action. Follicular growth is more marked and ovarian weights are greater than from the same dose of FSH given alone (Table II). This phenomenon of "synergism" or "augmentation" is shown best when both components are injected subcutaneously. The FSH and ICSH can, however, be given at separate subcutaneous sites (58), whereas in the nonspecific augmentation which results from injection of salts of heavy metals, the augmenting agent must be injected at the same site as the FSH. This suggests that nonspecific augmentors may change the rate of absorption of the FSH.

Higher doses of ICSH given subcutaneously with FSH not only augment the action of FSH on growth of follicles, but also promote the formation of corpora lutea (Table II). Luteinization can occur in follicles even when ova are not shed, in which case false corpora lutea

TABLE II
SYNERGISM BETWEEN PITUITARY FSH AND ICSH AT LOW AND HIGH DOSES IN HYPOPHYSECTOMIZED FEMALE RATS^a

Total dose in RU ^b		Ovaries			Histology		Uterus	
FSH	ICSH	Weight (mg.)	Follicles	Interstitial cells	Corpora lutea	Weight (mg.)	Description	
1/3	0	8	No enlargement	Deficient	None	21	Small	
0	1/5	0	No enlargement	Deficient	None	22	Small	
1/3	1/5	35	Small, medium, and large	Deficient	None	122	Estrous	
1	0	12	Small, few medium	Deficient	None	24	Small	
0	8	12	No enlargement	Repair	None	23	Small	
1	8	35	Large	Repair	Present	116	Estrous	
4	0	30	Medium and large	Deficient	None	122	Estrous	
0	4	17	No enlargement	Repair	None	25	Small	
4	4	55	Large	Repair	Present	138	Estrous	

^a Hypophysectomized at 20-28 days of age; one week postoperative, injected once daily, subcutaneously, for 3 days; autopsy 72 hours after the first injection.

^b RU = rat units.

are formed. Such false corpora are composed chiefly of luteinized thecal cells, and the granulosa and ovum eventually disappear from these structures. In normal corpus luteum formation, the granulosa as well as the theca is luteinized; in fact, granulosa lutein cells predominate in the normal corpora lutea of most species. It is not clear which hormone causes the epithelial transformation of the granulosa cells. There is some indication that the FSH itself is responsible, but it must be remembered that FSH has not been completely purified. Considerable confusion has been introduced into the literature by indiscriminate designation of true corpora lutea and thecal luteinized structures as "corpora lutea."

When combinations of FSH and ICSH are injected and the ICSH is given intraperitoneally, the action of the FSH is frequently found to be reduced instead of augmented. Whether this antagonizing of gonadotropic action is an intrinsic property of ICSH or is due to an accompanying factor, the "antagonist," is not known (69, 158).

Synergistic reactions have also been reported in the male following simultaneous injection of FSH and ICSH, leading to increased weights of testes and accessory organs beyond those resulting from injection of the separate components (57, 142). The phenomenon of synergism in the male has not, however, been as definitely established as in the female. The phenomenon of antagonism has not been reported in the male.

III. PROLACTIN AS A MEMBER OF THE GONADOTROPIC COMPLEX

A third pituitary hormone, which causes the corpus luteum to secrete progesterone, appears to be necessary for full reproductive function in the female. Corpora lutea in animals with very short cycles, such as the rat, do not become functional unless the animal breeds, in which case the corpora lutea persist and secrete progesterone. The life of the corpus luteum can also be prolonged and secretion of progesterone induced by cervical stimulation or by the injection of pituitary extracts. The preparation of the endometrium thus provided by progesterone in the fertilized animal allows implantation of the ovum. In the non-pregnant animal the proliferative state of the endometrium can be demonstrated by its ability to nidate threads inserted through the endometrium, resulting in placenta formation, a reaction useful as a test for corpus luteum function. Purified prolactin injected into an animal which has recently ovulated will also induce corpus luteum secretion and result in uterine responsiveness to stimulation (55, 56). The pituitary factor causing corpora lutea to function has therefore commonly been identified with prolactin. It is sometimes called the luteotropic hormone (4), a term not to be confused with luteinizing hormone. The

role of prolactin in inducing secretion of progesterone by the corpora lutea has not been established in many species. A substance with luteotropic properties similar to those of prolactin is present in the placenta of some animals (5). Chorionic gonadotropin has been reported to have a luteotropic action in women (15, 16). There is as yet no proven role of prolactin in the mammalian male.

IV. CHEMICAL FRACTIONATION OF PITUITARY GONADOTROPINS

Highly purified proteins, associated predominantly with one or the other gonadotropic action (follicle stimulation or repair of interstitial cells) and having widely different physicochemical properties, have been separated chemically from anterior pituitary substance and designated as the follicle-stimulating and interstitial cell-stimulating hormones. Whether the active principles are really proteins, or smaller units, such as polypeptides attached to specific proteins, has not been completely clarified. In fact, the concept of two separate pituitary gonadotropes as just defined is not entirely acceptable to all workers.

Further elucidation of the complex subject of the interrelation of the pituitary hormones and reproductive processes has been slowed by the nonavailability of pure hormones. In turn, the chemist has been handicapped by the necessity of relying on biological assays for identification of the hormones, and for determination of their freedom from biologically active and inactive contaminants. The chemical and physical means available enable the chemist to detect contaminants in the order of one-half to one per cent. The biological reactions can be elicited by much smaller amounts than the chemical, being exceeded in sensitivity only by certain of the immunological reactions. The biological end points for different hormones are, however, not of equal or of predictable sensitivity. The effective dose range of the pure hormone not being known, the chemist may pursue in his chemical manipulations the carrier protein and finish with a product which from physicochemical criteria could be regarded as homogeneous, and identified as the hormone by the biological assay, yet could consist of 99% inert protein and 1% hormone. Only with improvement of the chemical criteria can such erroneous conclusions be rectified.

Table III presents data showing the purity attained in chemical fractionations of the three pituitary gonadotropic hormones, as well as the pregnancy gonadotropins to be discussed later. The chemical attributes of these hormones are included, when known, and also the biological activity of the purified products.

are formed. Such false corpora are composed chiefly of luteinized thecal cells, and the granulosa and ovum eventually disappear from these structures. In normal corpus luteum formation, the granulosa as well as the theca is luteinized; in fact, granulosa lutein cells predominate in the normal corpora lutea of most species. It is not clear which hormone causes the epithelial transformation of the granulosa cells. There is some indication that the FSH itself is responsible, but it must be remembered that FSH has not been completely purified. Considerable confusion has been introduced into the literature by indiscriminate designation of true corpora lutea and thecal luteinized structures as "corpora lutea."

When combinations of FSH and ICSH are injected and the ICSH is given intraperitoneally, the action of the FSH is frequently found to be reduced instead of augmented. Whether this antagonizing of gonadotropic action is an intrinsic property of ICSH or is due to an accompanying factor, the "antagonist," is not known (69, 158).

Synergistic reactions have also been reported in the male following simultaneous injection of FSH and ICSH, leading to increased weights of testes and accessory organs beyond those resulting from injection of the separate components (57, 142). The phenomenon of synergism in the male has not, however, been as definitely established as in the female. The phenomenon of antagonism has not been reported in the male.

III. PROLACTIN AS A MEMBER OF THE GONADOTROPIC COMPLEX

A third pituitary hormone, which causes the corpus luteum to secrete progesterone, appears to be necessary for full reproductive function in the female. Corpora lutea in animals with very short cycles, such as the rat, do not become functional unless the animal breeds, in which case the corpora lutea persist and secrete progesterone. The life of the *corpus luteum* can also be prolonged and secretion of progesterone induced by cervical stimulation or by the injection of pituitary extracts. The preparation of the endometrium thus provided by progesterone in the fertilized animal allows implantation of the ovum. In the non-pregnant animal the proliferative state of the endometrium can be demonstrated by its ability to nidate threads inserted through the endometrium, resulting in placentoma formation, a reaction useful as a test for corpus luteum function. Purified prolactin injected into an animal which has recently ovulated will also induce corpus luteum secretion and result in uterine responsiveness to stimulation (55, 56). The pituitary factor causing corpora lutea to function has therefore commonly been identified with prolactin. It is sometimes called the luteotropic hormone (4), a term not to be confused with luteinizing hormone. The

role of prolactin in inducing secretion of progesterone by the corpora lutea has not been established in many species. A substance with luteotropic properties similar to those of prolactin is present in the placenta of some animals (5). Chorionic gonadotropin has been reported to have a luteotropic action in women (15, 16). There is as yet no proven role of prolactin in the mammalian male.

IV. CHEMICAL FRACTIONATION OF PITUITARY GONADOTROPINS

Highly purified proteins, associated predominantly with one or the other gonadotropic action (follicle stimulation or repair of interstitial cells) and having widely different physicochemical properties, have been separated chemically from anterior pituitary substance and designated as the follicle-stimulating and interstitial cell-stimulating hormones. Whether the active principles are really proteins, or smaller units, such as polypeptides attached to specific proteins, has not been completely clarified. In fact, the concept of two separate pituitary gonadotropes as just defined is not entirely acceptable to all workers.

Further elucidation of the complex subject of the interrelation of the pituitary hormones and reproductive processes has been slowed by the nonavailability of pure hormones. In turn, the chemist has been handicapped by the necessity of relying on biological assays for identification of the hormones, and for determination of their freedom from biologically active and inactive contaminants. The chemical and physical means available enable the chemist to detect contaminants in the order of one-half to one per cent. The biological reactions can be elicited by much smaller amounts than the chemical, being exceeded in sensitivity only by certain of the immunological reactions. The biological end points for different hormones are, however, not of equal or of predictable sensitivity. The effective dose range of the pure hormone not being known, the chemist may pursue in his chemical manipulations the carrier protein and finish with a product which from physicochemical criteria could be regarded as homogeneous, and identified as the hormone by the biological assay, yet could consist of 99% inert protein and 1% hormone. Only with improvement of the chemical criteria can such erroneous conclusions be rectified.

Table III presents data showing the purity attained in chemical fractionations of the three pituitary gonadotropic hormones, as well as the pregnancy gonadotropins to be discussed later. The chemical attributes of these hormones are included, when known, and also the biological activity of the purified products.

TABLE III
 PREBIOLOGICAL PROPERTIES OF CONJUGATED HORMONES

Chemical analyses (%)	Pituitary stimulating (FSH)		Intestinal cell stimulating (ICSH)		Prolactin		Equine serum (PMIS) (20, 150, 132)	Human chorionic (HCG) (25, 129)
	2log (79, 135, 152, 154, 161)	Sheep (94, 96, 100, 101)	2log (83, 98)	Sheep (104, 150)	Sheep (25, 96)	Beef (103)		
Nitrogen	15.1		14.9	14.2	15.9	14.4-16.8	10.0	11.7-12.6
Tyrosine	3.6		3.8	4.5	5.3	5.5-5.7	1.4	
Tryptophan				1.0	1.7	1.3-2.5		
Cystine	6-7	4.2	3.6	4.4	5.0	5.5	11.2-16.9	10-12
Carbohydrate hexose	3.5-4.3	1.3	2.3	5.6	5.6		5.6-12.8	5-6
Carbohydrate hexosamine	3.5-4.4	1.3	2.3	5.6	6.1			
Isoelectric point	4.8-4.8	4.5	7.45	4.0, 7.3	5.7	5.7	1.8-2.6	3.2-3.6
Molecular weight	29,000	87,000	100,000	31,000-40,000	24,500-26,500	32,000	30,000	100,000
Number of electrophoretic components	5-8	1	1	1-2	1	1	1	1-2
Mobility, cm/hour/100 v	-7, pH 7.5	-7, pH 7.8	+0.52, pH 7.6	-0.4, pH 7.5, +0.42, pH 7.1				+
Homogeneity ultracentrifuge	+	+		+	+	+		
Sedimentation constant $S_{20} \times 10^{13}$	6.8	4.7	6.6	2.4-3.6	2.1-2.2	5.6-2.8		
Enzyme digestion								
Pepsin	Unstable > 25%	Stable < 65% (dialyzable)	Unstable	Unstable	Unstable	Unstable	> 40-50%	
Crude trypsin	Relatively stable	Relatively stable	Unstable	Unstable	Unstable	Unstable	Unstable	
Crystalline trypsin	Unstable > 25%						> 40-50%	
Pyruvate or tanninase								
Solid-state pH and temperature	Unstable < pH 4, stable pH 3.2-10.2, 37° C, 4 hr.	Unstable pH 2, 30 min; stable pH 4, 50° C, 18 hr.			Stable pH 7.6, 100° C, 30 min. (in absence salt)		$\geq 50\%$ Inst. 40° C, 6 mo	
Biological activity	2.5 μ g (N 21 day female rat vagina-tation slope unit)	5 μ g (H 26 day female rat, follicular growth)	0.7 μ g (ventral prostate rat H 21 day)	5 μ g (H 26 day female rat IT repair)	25-40 IU/mg		50,000 IU/mg	8000-10,000 IU/mg

V. SPECIES SPECIFICITY IN PITUITARY GONADOTROPINS

A. Antihormones (*Antibody Formation*)

The nonspecies specificity of hormone action has been one of the basic concepts underlying endocrinology and this principle has been thought applicable to the gonadotropins. Table III shows that many of the physicochemical properties of the gonadotropins extracted from pituitaries are strikingly similar, although the hormones have been derived from different species. There is, furthermore, a striking parallel in biological effects of the gonadotropins in different species, and even in different vertebrate classes. The common experience is, in fact, that the gonadotropins are freely interchangeable in different species. Sheep gonadotropin causes ovulation in the cow. Gonadotropin prepared from human pituitary causes follicular growth and ovulation in the mouse. Sheep and hog pituitary gonadotropins cause follicular growth in the ovaries of monkeys and of human beings. Gonadotropins from beef, sheep, or pig pituitaries induce ovulation or spermiation in the frog and toad. Pituitary prolactin derived from mammalian sources not only induces mammary secretion in many mammalian forms but is highly effective in inducing secretion of food for the young (crop-milk) in pigeons.

The nonspecies specificity of gonadotropins has, however, been questioned on the basis of results from chronic injection of hormones from one species into another (31, 36, 52, 177). The initial stimulus from a gonadotropin from heterologous pituitaries has been noted in some instances to decrease or cease on continued injection, and the serum of such chronically injected animals, when injected with the gonadotropin into a second individual, has prevented the anticipated gonadotropic stimulation. This substance in the serum of chronically injected animals has not always been found to be associated with typical antibodies, such as precipitins or agglutinins. The phenomenon has therefore not usually been identified with true antibody formation, but has been designated antihormone formation. The development of inhibitory substances has in some instances been traced to the presence of contaminating proteins derived from the pituitary or serum of the source animal. The "antihormone" in some instances has not been specific in its action against the gonadotropin injected, but has also been able to inhibit the action of pituitary gonadotropins from other species or nonpituitary gonadotropic agents. The serum of chronically injected animals, instead of preventing the action of the administered gonadotropin, in some instances has been observed to increase its action in a second recipient. It should be borne in mind that the degree of purity of the hormones

injected has varied considerably, and very few, if any, entirely pure substances have been available for such studies. More investigation will be required in order to interpret satisfactorily the species specificity or nonspecificity of gonadotropins by the phenomenon of antihormone formation.

B. Species Differences in Biological Response to Gonadotropins

Other experimental evidence which has necessitated re-evaluation of the concept of nonspecies specificity of hormone action has arisen from certain instances of an initial failure of response in the target organ of one species when treated with pituitary "tropic" hormones derived from another species. Whereas the growth hormones derived from bovine, monkey, human, and whale pituitaries have all proved effective in the usual test animal for this hormone, the hypophysectomized rat (95, 126), it has been found that this nonspecificity of responsiveness is not true of other animals. Beef growth hormone has been shown to be effective in fish, but growth hormone derived from fish has not proved effective in mammals (169). The refractoriness of primates (monkey and man) to growth hormone derived from the usual animal source (beef or sheep pituitary) is well known, but an anabolic response to primate growth hormone has been obtained in a few instances (10, 85). With regard to gonadotropins, it has been found that primates usually give a limited response to pituitary preparations derived from nonprimate sources. FSH derived from pig or sheep pituitaries, or equine gonadotropin, readily induces follicular enlargement in ovaries of women and monkeys, but is inefficient in inducing ovulation. On the other hand, ovulation has been induced readily in monkeys by monkey pituitary preparations (165, 166). From recent chemical studies it appears that the species differences in chemical structure of hormones may involve a single amino acid, or single end group (45, 97). In some species these minute chemical differences appear to determine the biological response. Attention should again be drawn to the many observations showing that the hormones are usually interchangeable between many different species.

VI. REGULATION OF PRODUCTION AND SECRETION OF PITUITARY GONADOTROPINS

A. Action of Gonadal Steroids on the Pituitary

At least two mechanisms are known to be important in the regulation of the gonadotropic activity of the pituitary: the first, hormonal; the second, neural.

All of the gonadal steroids—estrogen, progesterone, and testosterone

—have been reported to inhibit gonadotropin production by the pituitary and, correspondingly, to lower the amount of circulating hormone (73, 136). The estrogens are by far the most effective. Even at low doses, estrogens restore normal cytology in the castrate's pituitary, reduce its elevated gonadotropic hormone content (125), and reduce the excess concentration of circulating gonadotropins. These effective doses of estrogen are well within the physiological range, being below the level necessary to cause cornification of vaginal epithelium or uterine stimulation (19, 20, 63). The effects of estrogen on pituitary production and secretion of gonadotropins can be demonstrated by direct bioassay of the pituitary and of the plasma. Parabiotically united rats are also well adapted for demonstration of this pituitary steroid interrelationship, since they reflect the serum hormone levels. Estrogen injected in small amounts into a castrate rat is able to inhibit the secretion of gonadotropin by the pituitary and so prevent the hypertrophy which otherwise would occur in the ovary of the normal (or hypophysectomized) parabiotic twin¹. Larger doses of either progesterone or androgens are required for this pituitary suppression.

B Direct Action of Gonadal Steroids on the Gonads

Testosterone propionate does not appear to be as effective in the female as estrogen in suppressing the gonadotropic activity of the pituitary, greater than physiological levels being required at least in some of the species studied (13). Activity in the testis of the rat can be suppressed by relatively low levels of testosterone propionate, presumably through pituitary suppression. Higher doses, however, stimulate testicular activity by direct action on the testis (105). This direct action of testosterone on the testis occurs in the absence of the pituitary. Local stimulation of the testis has been demonstrated in two species, the rat and monkey, the reaction being confined to the tubules adjacent to crystals of the androgen implanted in the testis (46, 148).

It should also be noted that estrogen has been shown to exert a direct action on the ovary, causing follicular stimulation in the hypophysectomized rat (170).

C Interpretation of Cyclic Reproductive Phenomena in the Female

The usual interpretation of cyclic reproductive phenomena in female mammals is based on alternating inhibition of pituitary gonadotropic

¹ This is interpreted as due to the persistence in the circulation of the gonadotropin from the castrate pituitary and its transmission into the parabiotically united animal, whereas estrogen is destroyed quickly by the liver and is not transferred in physiological quantities to the partner.

secretion by gonadal steroids, followed by reinstatement of secretion as the level of steroid falls. During the human menstrual cycle the gonadotropic content of the urine is higher at mid-interval. Follicular growth takes place during the first half of the cycle. At mid-cycle there is a preovulatory spurt in follicular growth rate, which probably corresponds in time to the mid-cycle increase in urinary gonadotropin. The larger follicles secrete increased amounts of estrogen, and it is assumed that when the estrogen content of the blood reaches a certain concentration the gonadotropic basophils of the pituitary are checked in production and secretion of FSH. Nothing is known regarding cyclic changes in the rate of production or secretion of the luteinizing factor, but the additional assumption is made that an increased secretion of ICSH (LH) occurs at mid-cycle, resulting in ovulation. It is known that estrogen injected under certain circumstances causes the outpouring of luteinizing hormone, and this may be the mechanism which normally completes the cycle (61, 84). Progesterone may check continued production and release of ICSH (LH). The control of prolactin secretion and its relation to these hormonal balances has not been elucidated.

D. Neural Control of Pituitary Gonadotropic Activity

Although ovulation in the rat was observed to be spontaneous, occurring rhythmically at a given phase of the estrous cycle, it was found subsequently that ovulation in some other species, e.g., in the rabbit, cat, and ferret, occurs only following breeding. The earlier concept that release of the luteinizing principle is hormonally controlled by interaction of pituitary and ovarian hormones therefore had to be modified, at least for some species, to include a neural release mechanism. It was found that ovulation in the rabbit did not follow copulation if the connection between the hypothalamus and the pituitary had been interrupted by severance of the infundibulum or by chemical blockage. Ovulation could, however, be induced in such animals by injection of a luteinizing hormone. These findings have been interpreted as showing that the failure to ovulate after severance of the infundibulum was due to interruption of an impulse, neural in origin, transmitted neurally or by the pituitary-portal vascular connections. In the absence of this neurohumoral stimulation the pituitary failed to secrete luteinizing hormone (or the so-called ovulation hormone). There is now reason to believe that even the rhythmic ovulation in the rat is mediated by hypothalamic stimulation, which causes the release of ovulatory hormone from the pituitary (59). In seasonal breeders, such as the deer, changes in intensity of light or seasonal additions of required food factors may be important in initiating the neurosecretory sequence in the pituitary.

VII. HORMONAL FACTORS NECESSARY FOR OVULATION

Although experimental induction of ovulation in rodents on injection of gonadotropins was demonstrated 30 years ago, the practical application of this knowledge has not been satisfactory. Although ovulation or superovulation has been induced in a number of species, the response has been found to be undependable. The incidence of ovulation has varied with the season in seasonal breeders, with the time in the cycle in animals with cyclic estrus, and with age, strain, and other undetermined factors. The effects of therapy have not always been distinguishable from spontaneous physiological changes or, in sterile animals, from spontaneous recovery (114, 115). Furthermore, induced ovulation frequently has not been coordinated with the occurrence of behavioral estrus, so that the animals would not breed. Gonadotropins have therefore proved of little value either in the breeding of farm animals or in solution of problems of human sterility. In experimental ovum transplantation in rodents, advantage has been taken of the multiple ova recovered after superovulation, and the capture of multiple ova from valuable farm animals for transplant and nurture in lower-grade foster mothers is of more than academic interest. There has also been some interest in efforts to induce ovulation at will in animals which otherwise would breed only seasonally.

Several recent reviews on the induction of ovulation in farm animals are available (28, 39). The earlier studies in sheep and goats were promising (33), but later work showed that the response was sporadic and fertility was low, due in part to lack of coordination between ovulation and estrus. In goats spontaneous ovulation of 4 or more ova has been reported following injection of 200–400 I.U. of equine gonadotropin (pregnant mare's serum, PMS) (64). In sheep, doses of 500 to 2000 I.U. of PMS resulted in the shedding of as many as 29 ova, and simultaneous administration of progesterone was reported to result in coincident estrus (133, 134).

Successful efforts to induce ovulation in cattle, both adults and calves, have been reported from injection of both PMS and pituitary gonadotropins. In cows, 3600–4500 I.U. of PMS followed by 2000 I.U. of human chorionic gonadotropin (HCG) resulted in the shedding of an average of 26 ova. When corpora lutea were present at the time of injection, the percentage of ovulations was higher (135). Gonadotropin from sheep pituitary, administered initially in wax pellets containing 1500 RU (rat units), followed 2 to 9 days later by a single intravenous saline injection containing 1000 RU, resulted in the liberation of as many as 38 ova within 24 hours after the intravenous injection. Similarly, pre-

liminary treatment with 1500 I.U. of PMS, followed 5 to 6 days later by sheep pituitary extract, resulted in superovulation (160). One or more ovulations resulted in over half of a group of calves injected for 3 to 4 days, subcutaneously, with anterior pituitary extracts (high in FSH), followed by HCG administered intravenously. As noted previously for adults, repetition of the treatment was more successful than the first attempt, and this again was attributed to the presence of corpora lutea (110). Ovulation in 80-94% of sows has been reported following injection of PMS during lactation, the uniformity of results being attributed to the stability of the physiological state (80).

Ovulation can be induced more reliably in several types of rodents. In the rabbit, a nonspontaneously ovulating animal in which growing follicles are constantly present, ovulation can be induced readily within a few hours by pituitary preparations or chorionic gonadotropin (the basis of the Friedman test for pregnancy). Immature mice and rats can be made to ovulate within 3 to 4 days by appropriate doses of either pituitary gonadotropic hormone, HCG or PMS. Mature mice have been reported to ovulate, irrespective of the time of the cycle, when injected with 1 I.U. of PMS, followed 40 hours later by 2 I.U. of HCG. Although superovulation resulted in 99% of these mice, estrous, mating, and pregnancy occurred in only 75%, and mean litter size did not exceed normal (67). In normal immature rats superovulation has been found to follow so reliably after injection of doses of 20 I.U. of PMS, followed 54 hours later by 20 I.U. of HCG, that these doses have been adopted as practical for study of the mechanism of ovulation and for collection of ova for artificial implantation (12). Although multiple ova, 37 or more, may be shed by the immature rat after such ovarian stimulation, this does not necessarily result in heightened fecundity; low rates of mating and fertilization, and frequency of fragmentation of eggs diminish the final numbers of young born. Such losses have been interpreted as due to poor hormone balance (6).

There have been a few reports of successful induction of ovulation in women (41). Isolated instances have been reported following injection of pituitary extracts and PMS: equine gonadotropin (3000 I.U.) supplemented by human chorionic gonadotropin (38,000 I.U.) has also been successful (25). Pituitary gonadotropins have been tested in women only infrequently, due in part to their unavailability and in part to inadvisability in view of possible allergic reactions or immune body formation (109). In the monkey, ovulation has been reported to occur sporadically after injection of sheep or pig pituitary fractions, or of PMS (78, 83). Recently ovulation has been reported to follow more

consistently after administration of gonadotropins from monkey pituitary than after sheep gonadotropic fractions (165, 166).

The studies on induced ovulation reported above were all conducted in individuals possessing a pituitary which may have contributed to the response. Until ovulation can be induced regularly in the absence of the pituitary it is impossible to determine accurately the hormonal requirements for ovulation. Recently ovulation has been induced in the hypophysectomized rat by pituitary preparations consisting almost exclusively of FSH, contaminated by only small amounts of ICSH (21). In such experiments the test animals were given a total dose of 4 RU of FSH in 4 days, followed by an ovulatory dose of 8 RU subcutaneously at the end of the fourth day. As determined by bioassay, the amount of ICSH contained in the total dose was approximately 1 RU. Ovulation followed this treatment regularly within 18 to 24 hours, and as many as 65 ova were shed. Corpora lutea so induced could be brought to functional state by injection of prolactin, as judged by the placentoma test.

VIII. HORMONAL FACTORS NECESSARY FOR ESTABLISHMENT AND MAINTENANCE OF PREGNANCY

The relative importance of hypophyseal, ovarian, and placental hormones in the maintenance of pregnancy has been studied in very few species (127, 149). The pituitary has been shown to be dispensable in the latter part of pregnancy in both rats and monkeys. The pituitary has been removed from rhesus monkeys between days 32 and 106 of pregnancy, yet gestation continued to parturition between the 149th and 186th days; labor was prolonged and lactation did not occur. The hormonal requirements necessary for maintenance of pregnancy have been determined accurately in the rat. The pituitary of the rat can be removed without interruption of pregnancy after the 11th or 12th day. By this period the placenta is sufficiently developed so that it, together with the ovary, can furnish the necessary hormonal support. Placental luteotropin appears to be the factor capable of replacing prolactin in later pregnancy (131). The ovary is necessary throughout pregnancy in the rat unless estrogen and progesterone are provided in proper proportions (1 μ g. and 4 mg. daily) (108).

Hypophysectomized rats allowed to go to term by using their own placental and ovarian hormones, or maintained after ovariectomy by estrogen and progesterone, frequently experience difficulty in parturition, with prolonged labor. Death often ensues, due in part to exhaustion, the hypophysectomized rat being poorly equipped to maintain

liver glycogen and hence blood sugar level during prolonged effort. When pituitary therapy or progesterone is administered in the effort to maintain the level of steroids necessary for maintenance of pregnancy, the prolonged action of progesterone may be the cause of the lengthening of pregnancy. The tenet that a decrease in the progesterone level precipitates labor is not universally accepted (66, 175).

Hypophysectomized rats are unable to suckle their young (whether born or surgically removed). Several factors appear to be essential to the hypophysectomized rat for milk production and milk flow. Estrogen and progesterone are necessary to build up the mammary tree, prolactin to cause lobulo-alveolar development with milk production. In addition, some support from pituitary growth hormone, possibly also from thyroxine and from adrenal steroids, directly, or via their respective tropic hormones from the pituitary, is necessary for normal milk secretion. As posterior lobe hormone (oxytocin) is reported to be necessary for the discharge of milk, and as the posterior pituitary is removed by the usual procedure employed in hypophysectomy in the rat, this factor also may be involved in the failure of lactation in hypophysectomized animals.

The striking differences between species in degree of dependence on the ovary during pregnancy are exemplified by differences between the rat and primates. Whereas in the rat the ovary is necessary throughout pregnancy unless substituted by injection of ovarian hormones, in the primate the ovaries are dispensable early in pregnancy, as soon as the placenta is well established. In human pregnancy the ovary is dispensable by the time of the second missed menstruation, and in the monkey the ovary can also be removed early in pregnancy without interrupting it. Thus, in the primates the placenta appears to be able to produce the necessary ovarian hormones far more adequately than in rats.

The ovarian, pituitary, and placental requirements for maintenance of pregnancy in different species, the hormonal contribution of the fetus itself, and the factors involved in parturition and lactation are more fully considered in other chapters of this volume.

IX. HORMONAL INTERRELATIONS IN PROBLEMS OF FERTILITY

The complex hormonal interrelations which must be coordinated in order to insure fertility are reviewed in diagram form in Fig. 17. Briefly, the diagram shows that in the male, one, or perhaps two pituitary gonadotropins, ICSH and possibly FSH, are necessary for the multiplication of immature germ cells, for the growth of spermatocytes and

their meiotic division, and for the formation and maturation of the spermatid and release of mature spermatozoa. The Leydig cells must be stimulated by ICSH to produce testosterone, which in turn develops the accessory reproductive system and so ensures the formation of seminal fluid and transport of sperm from the testis to the urethra. In man it requires 10 days for the sperm to pass through the entire length of the epididymal duct, and during this time they continue their maturation.

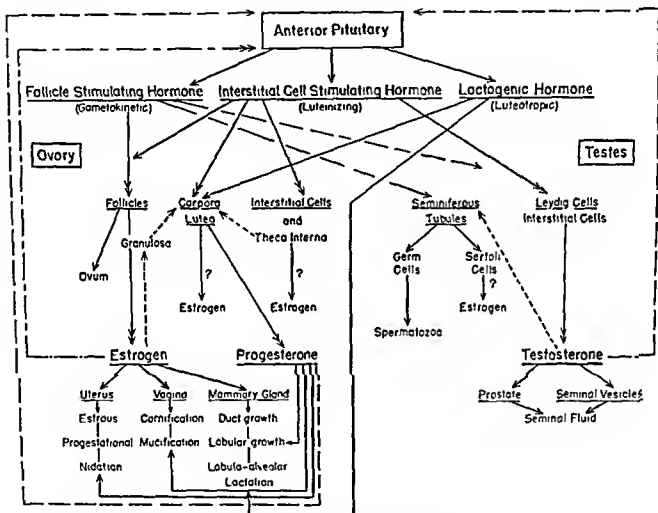


FIG. 17. Summary of interrelations between the anterior pituitary and the reproductive system. Modified after Simpson (138).

tion, provided they are adequately supported by this organ. The complexity of the process is obvious when one considers the number of glands between the testis and urethra which contribute to the formation of normal seminal fluid.

In the female, the interrelations between hormones and the timing of their actions is even more intricate. Pituitary FSH is necessary for growth of follicles and maturation of the ova. FSH is undoubtedly synergized in this action by ICSH. ICSH is probably also necessary for ovulation and formation of the corpus luteum. Prolactin is needed for

function of the corpora lutea. The secretion of estrogen by the follicle, and perhaps also by atretic follicles (156), interstitial cells, and the corpora lutea is necessary for growth of the remainder of the female reproductive system—uterus and tubes, vagina, and mammary glands. Progesterone is needed to complete the endometrial proliferation and glandular secretion in preparation for nidation of the embryo and for arborization of the mammary gland. The gonadal steroids exert a reflex control on gonadal activity by impeding or checking pituitary secretion of gonadotropins in females as well as in males. In this control of the pituitary, and hence of the gonads, the importance of hypothalamic neural mechanisms is gradually becoming understood.

The ovum, after expulsion from the ovary, must be picked up at once by the infundibulum and transported along the oviduct at the proper rate by ciliary and muscular activity. Fertilization in the oviduct is possible within a very narrow limit of time. On entry into the uterus the blastocyst will survive only if the endometrium has been properly prepared by estrogen and progesterone. All hormonal factors must be present at optimal concentrations and at the proper time to ensure completion of these processes. Infertility will result from lack of integration of any phase of this complicated hormonal sequence. Mention has already been made of the difficulties which have been experienced in efforts to reproduce the correct sequence and balance of pituitary and ovarian hormonal action in order to ensure ovulation and concurrent estrus so that insemination will result. The Conference on Sterility and Fertility held in Tokyo in 1955 dealt extensively with these problems of coordination, and its reports contain many valuable reviews (123).

X. DIETARY-HORMONAL INTERRELATIONS IN REPRODUCTION

A relation between diet and reproductive performance has been known from empirical observations for many years. With the growth of our knowledge of endocrinology and nutrition, the interactions between essential endogenous and exogenous factors in reproduction are beginning to be better understood. It is frequently difficult to distinguish between the effects of deficiencies in specific food factors and those of caloric or food restriction; both usually lead to a secondary protein deficiency. The resulting disturbances in reproductive function are usually reversible by supplying a proper diet or by injection of gonadal or gonadotropic hormones. The atrophic condition occurring in general malnutrition has been termed pseudohypophysectomy, from the close resemblance of the changes in many of the endocrine organs to those following hypophysectomy (113).

Deficiency in the male rat of any of the many factors known to be essential in this species, with the exception of vitamin E (48), has been shown to result in atrophy of the accessory organs, followed by decreased testicular weight (65). The primary change in the testis was found to be atrophy of the interstitial tissue. Vitamin E deficiency, on the other hand, causes no defect in the Leydig tissue and no atrophy of the accessory male system, whereas the seminiferous tubules show irreversible degeneration. Changes in the anterior pituitary occurring in vitamin E deficiency are similar to those following castration (86, 163).²

In the female rat, the disturbances resulting from lack of a specific food factor are frequently characterized by disturbances in reproductive function. Pteroylglutamic acid deficiency in many species of both mammals and birds has been found to be characterized by decreased response to injected estrogen (81, 82). Deficiencies of protein, thiamine, or riboflavin, on the other hand, are characterized by increased and prolonged response to injected estrogens, apparently resulting from reduced liver inactivation of circulating estrogens (47, 81, 82). The reproductive disturbances, including loss of the estrous cycle and fetal death, which follow deficiency in protein, potassium, pyridoxine or thiamine, appear to result from interference with maternal production and secretion of sex hormones (11, 116, 118, 119, 121). Pregnancy can be maintained in the presence of each of these four deficiencies by daily injections of estrogen and progesterone, the doses being the same as those necessary for maintenance of pregnancy in the absence of the pituitary or the ovary (108). Pituitary bioassays have shown that neither in B₆ deficiency (174) nor in the absence of dietary protein is the gonadotropic content of the pituitary reduced; instead, it is significantly increased (151). The defect may, therefore, be either in release of hormone from the pituitary or in ovarian response to gonadotropins.

In deficiencies of vitamin A, E, B₁₂, riboflavin, pantothenic acid, or pteroylglutamic acid, reproductive failure is associated with fetal death or congenital abnormalities (117). In these deficiencies the mothers appear only slightly affected, and there appears to be no lack of sex hormones, since such substitution therapy will not enable the pregnancy to continue (120). The embryos undergo abnormal development or

² Castration changes in the pituitary in the presence of normal testicular Leydig cells and evidence of testosterone secretion in maintenance of the male accessory organs are not in keeping with the concept that the gonadal steroids regulate pituitary secretion of gonadotropins.

die, from which it appears that these essential dietary factors play a key role in embryonic differentiation and organogenesis.

XI. GONADOTROPINS IN BODY FLUIDS

A. *Gonadotropins in Body Fluids During Pregnancy*

Gonadotropic substances have been described in blood and urine in only a few species and under limited physiological conditions. In two instances the gonadotropic substances are known definitely not to be of pituitary origin, namely, the gonadotropin found in the bloodstream of pregnant equidae (horses, zebra, asses) and that found in the blood and urine of pregnant women (and in other primates, such as the rhesus monkey, orangutan, chimpanzee).

The equine or pregnant mare's serum gonadotropin (PMS) appears in the bloodstream of the mare (32) in large quantities early in pregnancy (70th to the 120th day), but its concentration in the urine is low (Fig. 18). In ponies, 200 I.U. per milliliter are frequently found in the blood and 1/5 I.U. per milliliter in the urine; if the ponies are pregnant and lactating, the milk contains 1/5 I.U. per milliliter (Cole, unpublished). It is reported to be derived from cuplike structures in the endometrium rather than from the chorion (26, 29). This gonadotropin exerts follicle-stimulating, interstitial-stimulating, and luteinizing action in normal as well as hypophysectomized animals. It has been prepared in highly purified form, 30,000 I.U./mg. (99, 130, 132), yet it has not been altered (30) at any stage of purification in its chemical characteristics and biological effects (Table III).

The hormone characteristic of human pregnancy is present in both blood serum and urine. It originates from the syncytial layer of the chorionic villi (71), and is therefore designated human chorionic gonadotropin (HCG). It, too, has been highly concentrated and purified (8000-10,000 I.U./mg., Table III). At all stages of purification it retains its capability to promote the development of follicles and corpora lutea in the immature female rat. In the hypophysectomized rat its typical effect is the stimulation of the interstitial cells. When this substance is injected simultaneously with pituitary FSH into the hypophysectomized rat, the ovary resembles that of the normal adult; fully developed follicles and corpora lutea are formed (51, 53, 93). The ovarian weights resulting from injection of FSH with HCG are greater than anticipated from the gonadotropic action of the individual components, whether tested in hypophysectomized or normal immature rats. As is the case with ICSH, HCG synergizes the action of FSH on follicular development.

At extremely high doses, preparations made from human pregnancy urine cause limited follicular stimulation in hypophysectomized rats; this has often been attributed to the presence of small amounts of a follicle-stimulating substance (106). Whether the follicular stimulation is due to the presence in urine of small amounts of pituitary FSH, or is an intrinsic property of the chorionic hormone has not been determined, although there are a few reports indicating the chemical separation of two factors from pregnancy urine (44, 129, 137). The presence of de-

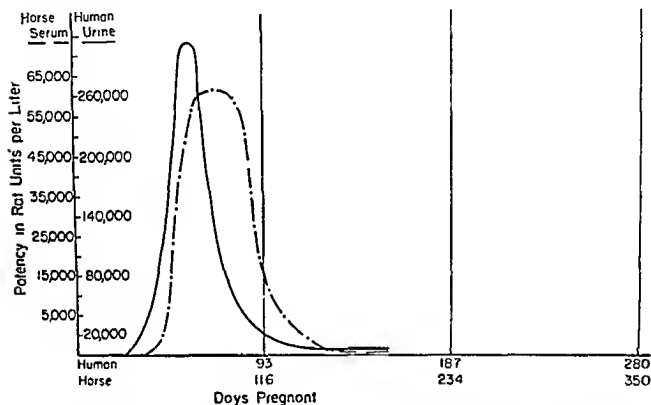


FIG. 18. Gonadotropic hormone titers in pregnant mare's serum and human pregnancy urine. Scale for thirds of pregnancy in the horse superimposed on that for trimesters in the human. Modified after Cole and Saunders (34); Evans *et al.* (50).

teable amounts of pituitary FSH in blood or urine during pregnancy seems unlikely in view of the markedly diminished gonadotropic hormone content of the human pituitary during pregnancy, (17, 128).

B. Detection of Pregnancy by Bioassays of Serum and Urine

The discovery of gonadotropin in the blood and urine during pregnancy has furnished a valuable index of pregnancy in those forms in which gonadotropin is found, at least during those periods in which hormone continues to be secreted. The test for pregnancy in women is commonly applicable from approximately the time of the first missed menstruation to term. The hormone can actually be detected as early

as 16 days after a known day of insemination. The highest titer is reached during the first trimester, about 35 to 45 days after ovulation or 50-60 days after the last menstrual period (Fig. 18). In primates other than man, the hormone is present in the urine for only a short period, so that its detection is correspondingly less valuable as a test for pregnancy. In the rhesus monkey, it is detectable by the usual methods only between day 22 and 34 of pregnancy and is present only in low titer (77, 164). In the mare, where the hormone is found only in the blood plasma, the test is applicable from about the 40th day of pregnancy through the first third of gestation (Fig. 18). Gonadotropins have not proved to be present in increased quantities during pregnancy in other mammalia. It is possible that substances characteristic of pregnancy may be detected in plasma or urine of other mammals when advantage is taken of the augmentation reaction with FSH, and this reaction may eventually prove of value as a pregnancy test. Such substances have been shown in body fluids of the pregnant cow and guinea pig (54, 92). Increased excretion of estrogen and progesterone, or their derivatives, may also extend the range of hormonal assays for pregnancy.

C. Gonadotropins in Body Fluids of Nonpregnant Animals

Gonadotropins have been reported only rarely in the body fluids of nonpregnant animals. Barely detectable amounts have been reported in the blood plasma of rats after castration, but not in normal rat plasma (37, 38, 40). Small amounts of gonadotropic hormone are present in the blood plasma and urine of nonpregnant women, and in men; these hormones are assumed to be of pituitary origin (7, 8, 9). Increased amounts of gonadotropic hormone appear in the blood and urine of women after the menopause or after castration. Assayed in normal or hypophysectomized rats, the urinary hormone of menopause or castration causes follicular growth and at high doses causes luteinization; in the hypophysectomized rat it repairs the deficient interstitial tissue.

XII. BIOASSAY OF GONADOTROPINS

Many bioassays have been devised for the gonadotropins and the subject has been reviewed frequently (24, 42, 52, 75, 76, 103, 104). The reliability of each test depends upon its adaptability to the biological properties of the particular gonadotropic agent to be tested, and upon the use of sufficient numbers of appropriate experimental animals under controlled and constant conditions. The choice often depends upon the convenience in the particular laboratory, the facilities available, and the training of the staff. The particular assay method chosen is not of

such crucial importance if an international standard preparation is available, permitting the expression of results in terms of an international unit for the hormone in question. The only gonadotropic agents for which international standards have been adopted are the gonadotropin in pregnant mare's serum, and that in the blood and urine of pregnant women (88, 89). Lactogenic hormone, or prolactin, also has an international standard and unit (90). In the case of all other gonadotropins, including those extracted from the pituitary, and the hormones which are assumed to be derived from the pituitary (those found in the body fluids of children, nonpregnant young women, postmenopausal women, normal men, and castrates of both sexes), no international standards have been adopted. There is, furthermore, no satisfactory agreement regarding the reliability of the proposed tests, the interpretation of these tests, or even the hormones measured by the assays.

A Assay of Pituitary FSH

Table IV lists a few of the more satisfactory assays for pituitary FSH. The test in the hypophysectomized immature female rat is placed first as giving the most reliable assay for FSH, both for potency and for detection of biologically active contaminants (58, 102). One of the great advantages of this test is that it can also be used for assay of the second pituitary gonadotropin, ICSH, and for determination of the proportions of FSH and ICSH in a mixture. Furthermore, it can be used for simultaneous detection of all the other tropic hormones of the anterior lobe, with the exception of prolactin³. In the assay using hypophysectomized rats, the minimal dose which, when given subcutaneously, reinstates microscopically detectable increases in follicular size is taken as the unit for FSH. Evidence of contamination of FSH preparations by ICSH can be ascertained by the repair of the interstitial cells; greater contamination by ICSH elicits corpus luteum formation. In the interpretation of the test it is essential to remember that in the presence of ICSH the quantity of FSH present, judged by the number and size of follicles, is increased (phenomenon of synergism). Conversely, when more highly purified FSH preparations are tested, the potency judged by the degree of follicular growth appears to diminish with increased purification, i.e., removal of ICSH. The objections to

³ The content of growth, thyrotropic, and adrenocorticotrophic hormones can be measured in the same 3- or 4 day test. By the use of properly prepared hypophysectomized rats the test can also be adapted to the simultaneous assay of prolactin by the placenta test (55, 56), however, this assay is far less sensitive than the pigeon crop assay method (107).

TABLE IV

ASSAYS FOR PITUITARY GONADOTROPINS: FSH, ICSH, PROLACTIN (52, 55, 58, 107, 140, 155, 173)			MED, ^a mg.	
Hormone	Procedure	Criteria	MED, ^a mg.	
FSH	Hypophysectomized 20-28-day female rats, 6-8 days postoperative; inject once daily 3 days, subcutaneous, 72-hour test	MED (histological) incipient follicular development: increase of small to medium follicles	0.003	
	Normal 24-25-day female rats; augmentation test; inject once daily 3 days, mixed with HCG (alone 35-40 mg. ovaries)	100% increase ovarian weight	0.003	
	Normal 21-22-day female rats; augmentation test; inject as above	Slope units regression curve	— ^b	
	Hypophysectomized 20-28-day female rats, 6-8 days postoperative; inject once daily 3 days, IP, 72-hour test (subcutaneous 1/5 as sensitive)	MED histological evidence for repair of deficient IT	0.005	
ICSH	Same in hypophysectomized immature male rats	Repair of Leydig cells	0.005	
	Hypophysectomized 21-22-day male rats, 2 days postoperative; inject 4 days IP	> 70% increase ventral prostate weight	0.050	
	Augmentation in 20-28-day hypophysectomized female rats, 6-8 days postoperative; inject once daily, 3 days mixed with 2-3 RU FSH subcutaneous	> 100% increase total accessory weight	0.100	
	Nonnull 24-25-day female rats; unknown injected with 2-4 RU FSH	Histological: Increase in size of follicles above that produced by FSH alone; estrous uterus	0.001	
Prolactin	Local crop unit (immature pigeon)	Increase in follicular size, and corpus luteum formation.	— ^c	
	Systemic crop unit (immature pigeon)	Local crop sac epithelial response	0.0001	
	Placentoma test (hypophysectomized rat)	Thickening of epithelium and crop weight increase	0.001	
		Uterine deciduoma	0.060	

^a MED stated when comparisons could be made from the same preparation of a given gonadotropin.

^b In terms of slope units, 850 SU in 25-day-old rats, or 660 SU in 21-day rats, have been found to be equivalent to 1 augmentation unit or 2 hypophysectomized rat units as defined above.

^c A different ICSH preparation with MED 12.5 µg.; effective range in augmentation 2-4 RU.

the test are that it requires hypophysectomized rats and special facilities for their maintenance, as well as a staff for the preparation and study of histological sections. If such requirements can be met, this is the method of choice for determining the gonadotropic components present in the preparation tested, and the amounts and proportion of each.

Other tests for FSH are listed in Table IV in order of decreasing sensitivity or reliability. The augmentation of ovarian weights in normal immature rats resulting when FSH is combined with a luteinizing agent (ICSH, or preferably HCG) has proved a valuable and simple test for FSH. This assay is of approximately the same sensitivity as the hypophysectomized rat test for FSH, when 100% increase in ovarian weight resulting from the combination is taken as the unit (58). Recently the augmentation test has been given statistical reevaluation. When the unitage is thus calculated in slope units on a regression curve the unit is much smaller (155). The augmentation assay using normal immature rats has greater value in the determination of increased potency of FSH preparations during purification procedures, rather than in the quantitative determination of the biologically active contaminants. As in other tests in which animals possessing pituitaries are used, the augmentation tests do not give as accurate a measure of the FSH as is given by the hypophysectomized rat. The response measured is influenced not only by the augmenting or antagonizing effects of the contaminants present in each of the two preparations injected, but also by contributions from the pituitary of the assay animal (hormones secreted normally by the pituitary, as well as those secreted in increased amounts under the stimulus of the substance injected). Assays based on increase in uterine weight in normal immature rats or mice likewise involve the interaction of the test animal's pituitary. Such assays are subject to the further criticism that purer FSH preparations must be given in higher multiples of the minimal effective dose, because of removal of ICSH during purification and therefore the elimination of its augmenting action, and hence reduction of estrogen production.

B. Assay of Pituitary ICSH

The method of choice for assay of pituitary ICSH is, for similar reasons, that based on the response in hypophysectomized immature female rats (Table IV) (74, 140). The smallest dose which initiates repair of the deficient interstitial tissue is determined by intraperitoneal administration of a series of diminishing doses. The degree of contamination of ICSH preparations with FSH can best be determined by subcutaneous administration. In the presence of large amounts of

FSH in a preparation, particular care is required in the evaluation of the ICSH. The repair of Leydig cells in the testis appears to be about equally as sensitive a measure of ICSH as is that of the interstitial tissue in the ovary. A more popular test, employing the hypophysectomized rat but requiring no histological evaluation, is based on the weight increase of the ventral lobe of the prostate of hypophysectomized 21-day-old rats. A 70 or 100% increase has been used commonly as the end point, although recently interpolation on a regression curve has frequently been adopted, in some instances dangerously small increments being regarded as significant. An increase in total prostate or total accessory organ weight can also be used satisfactorily (43). The augmentation test in normal or hypophysectomized immature female rats can be used in measurement of ICSH, in which case the augmenting agent added is FSH. In any tests for ICSH in which the normal immature rat is used, the test is subject to the same criticisms discussed previously in relation to assay of FSH.

C. Assay of Human Chorionic Gonadotropin (HCG)

In assays of gonadotropins in body fluids, as in assays of pituitary hormones, the hypophysectomized rat gives the most accurate measure of the hormones present, whether expressed in terms of the absolute amounts or in terms of the proportion of FSH-like and ICSH-like properties. As pointed out in the description of the biological properties of gonadotropins in body fluids, the ICSH-like properties of the hormone in human pregnancy predominate so greatly that very high doses must be given before any capacity to stimulate follicles can be demonstrated. On the other hand, injection of HCG in intact immature rats readily induces follicular growth and corpus luteum formation, and this response in the normal immature rat or mouse forms the basis for the assay of HCG and for the earliest described pregnancy test, the Aschheim-Zondek (A-Z) test (3).

The methods adopted for assay of HCG have multiplied greatly because of the interest in rapid, simple, accurate, and cheap methods for detection of early pregnancy. Many excellent reviews on these tests are available (14, 18, 42, 43, 72). Table V compares several such tests. As HCG is one of the few substances for which an international standard has been adopted, any of these tests can be interpreted in terms of the international unit (I.U.). On the whole it can be said that the original A-Z test is as accurate as any. However, it requires a test period of 3 to 4 days, which accounts for the popularity of the more rapid tests which are in common use, although often less sensitive and less accurate.

TABLE V
ASSAYS FOR HUMAN CHORIONIC GONADOTROPIN (2, 14, 18)

Test animals	End point	I U	Per cent accuracy	Relative cost	Time required (hours)
Immature female rat (A-Z)	Corpora lutea, 50%	1.1	99	4	96
Immature female mouse (A-Z)	Corpora lutea	2.5			
Uterus, rat	Weight, 50 mg	0.3			
Vagina, rat	Cornification, 50%	0.4			
Toads or frogs, male	Spermiation	1 to 4	85	1	1-4
Rabbits, infantile or adult females	Ovulation	5	98.5	10	24
Immature female rats	Ovarian hyperemia	20	69		2
			99.5		24
Hypophysectomized male rats	Ventral prostate weight, total accessory weight			20	96

The 2-hour spermiation test in frogs or toads is in this category, as is the ovarian hyperemia test. Although a period of 2 to 4 hours is often used in the hyperemia test, its accuracy increases with longer periods (2, 78, 157).

The interpretation of these various assay methods for HCG can not always be made in terms of the intrinsic properties of the hormone, as contrasted with effects secondary to pituitary stimulation. The spermiation test in amphibia appears to be a function of the ICSH-like properties. The A-Z test in immature female rodents is secondary to pituitary stimulation, the resultant ovarian reaction representing the action of the secreted pituitary component augmented by the intrinsic luteinizing properties of the substance injected. Although the rabbit ovulation test (Friedman's) may depend primarily on the luteinizing properties of HCG, an augmentation effect dependent upon the recipient's pituitary FSH may contribute to the final impulse to preovulatory growth of follicles. In the hyperemia test the luteinizing properties are thought to be most important, but it is known that the reaction can be augmented by addition of FSH (70, 157).

D. Assay of Equine Gonadotropin (PMS)

Any one of the well-established tests for potency of PMS is adequate, particularly if comparisons are made with the international standard (Table VI). The most commonly used assay calls for a direct measurement of ovarian weight increment, followed by interpolation in the standard dose-response curve (usually in the steepest part of the curve, between 10 and 50 I.U. (22, 32). The secondary response of the uterus

TABLE VI
ASSAY OF PREGNANT MARE SERUM GONADOTROPIN (22, 75)*

Procedure	Criteria	Effective dose range (I.U.)
Hypophysectomized 26-28-day female rats 6-8 days postoperative; inject once, or daily 3 days, IP; autopsy, 72 hours	MED for follicular development	2-5
	MED for interstitial tissue repair	1-2
Normal 21-25-day female rats; inject once, or daily 3 or 4 days subcutaneous, or IP; autopsy 72 or 96 hours	Ovarian weight increase ^b	(4-20)
	Corpora lutea	1.0-4.0
	Uterine weight increase	0.25-1.0
	Vaginal cornification	0.20-2.5
Normal immature mice	Ovarian weight increase ^b	(2-10)

* Personal communication.

^b Interpolation in steepest part of dose-response curve.

or vagina can also be used for assay purposes. In the male, the secondary response of the accessory organs (ventral prostate, seminal vesicles, or total prostate) is also practicable in measuring PMS, due to the sensitive response of the Leydig cells to this hormone.

E Assay of Urinary Gonadotropins in Nonpregnant Human Beings

Gonadotropins appear in the blood and urine of nonpregnant individuals only in low concentrations⁴. Such hormones in body fluids are assumed to be derived from the pituitary. Due to the small amounts present these gonadotropins are difficult to concentrate and detoxify sufficiently for assay. No one has isolated the pure gonadotropins from these sources or fractionated them into follicle-stimulating and luteinizing hormones, as has been done for the anterior pituitary gonadotropin. Furthermore, these hormones from body fluids have not been thoroughly characterized by assay in hypophysectomized animals. Some study has been devoted to the physiological properties of the substances in urine of normal men and of postmenopausal women and of castrates (49, 76a, 91). The dominant action of the latter hormones is that of promoting follicular growth, although they stimulate interstitial cells and cause corpus luteum formation when given at high dose levels. Not only is the total potency per unit volume increased in urine of postmenopausal women and from castrates of both sexes, but the FSH-like property disproportionately so, as compared with values obtained from the urine of normal men (see Table VIII). Less well documented is the report that the urinary hormone obtained from young women is predominantly FSH like in its action.

A number of assays have been used in attempts to measure the gonadotropic potency of such urinary hormones. The multiplicity of tests is attributable in part to the difficulties of such assays because of the small amounts present and hence the large volumes of urine which must be concentrated for injection. To measure the FSH-like potency of the specimen the following end points have been recommended: (1) response of the ovary, preferably of the hypophysectomized rat, (2) the secondary uterine weight increase, and (3) the augmentation

⁴ This statement is exclusive of human patients having certain types of tumors in the female, chorioepithelioma or hydatidiform mole originating in the uterus following pregnancy, in the male, chorioepithelioma and teratomata originating in the testis. In both men and women with such tumors extremely high titers of gonadotropin appear in blood and urine. The hormone is usually identified with human chorionic gonadotropin. It originates in tumor cells from modified chorionic tissue in the female and in the male from tissue strikingly resembling trophoblastic tissue.

of the ovarian response to HCG. The test based on increase in weight of the mouse uterus is, perhaps, used most frequently, as this test is simple to conduct and the response is several fold more sensitive than the rat uterine or ovarian response.

When it is desired to determine the ICSH-like potency of the urinary product, one may use either the test based upon the repair of interstitial cells in the hypophysectomized rat, or the test based upon the weight increment of the ventral prostate in hypophysectomized (or normal) rats (74, 112). The augmentation of ovarian weights on combination of urinary concentrates with FSH has enjoyed only limited use as a means of detecting luteinizing substances. The hyperemia test when applied to such specimens can probably be placed justifiably in the category of tests which measure luteinizing potency. Any assays for ICSH-like potency carried out on normal animals are subject to the same limitations as were discussed under assays for pituitary and pregnancy hormones. The interpretation of all assay methods for gonadotropins in body fluids has been confused by the free use of the term FSH for any urinary product initiating follicular growth, and the term ICSH (or LH) for any fraction causing interstitial repair or corpus luteum formation, even though the identity of these substances with the analogous hormones extracted from the pituitary has by no means been established.

The gonadotropic titers found in urines from various human sources have been listed in Table VII. The values cited are all from assays based on the mouse uterine test, since more data are available from this than from any other single test. In evaluating the mouse uterine assay it can be said that those urines which have a predominantly follicle-stimulating action, namely, the urinary hormones found in castrates and menopausal women, also cause the most marked uterine enlargement. In such evaluations of urinary FSH-like substances based upon uterine reactions elicited from normal rodents, it should be remembered that some stimulation by ICSH or ICSH-like substances may be required for follicular growth and secretion of sufficient estrogen to cause, in turn, uterine enlargement and secretion. Also, it is well to remember, in using the uterine response of intact animals, that the pituitary of the assay animal may also contribute to the reaction. Human chorionic gonadotropin, a urinary hormone almost exclusively ICSH-like in its properties, judged by the hypophysectomized animal, can be, and often is measured by its ability to induce estrous uterine (or vaginal) reactions in normal immature rats or mice. The ovarian response of the hypophysectomized rat gives a more precise measure of the FSH-like or ICSH-like substances present. Every urinary hormone so far examined has

been shown to possess some ICSH-like properties when tested at appropriate doses. Although interstitial cell-stimulating substances occur in varying amounts in urines of nonpregnant human beings, the titer is usually low, markedly so when compared with pregnancy gonadotropins. In no instances other than pregnancy have such interstitial cell-stimulating substances been found free of FSH-like properties.

TABLE VII
GONADOTROPIC HORMONE TITERS IN HUMAN URINE (52)^a

Status	MUU per 24 hours, average
Prepuberal	≤ 3-5
Young adult women,	
Early and late menstrual cycle	5-7
Mid-cycle	20-50
Adult men	20-40
Postmenopausal women and castrates	50-100
Hypogonadism	
Pituitary origin	3-7
Gonadal origin	50-200

^a Assayed in 21-day female, nonpregnant, nontumor-bearing mice, injected subcutaneously daily for 3 days; autopsy at 72 hours. Increase in uterine weight, 100%.

Many difficulties are encountered when one attempts to compare the values of urinary assays obtained from different laboratories, even when the values are based upon the same assay method, as, for example, the mouse uterine weight assay. Methods for concentration of the urine vary; these methods are not equally efficient, and furthermore, some of the extracts are toxic at the high concentrations which must be injected. The end points used also differ, some being based on 100 or 200% increase in uterine weight, and others, more recently, on interpolation in a dose-response curve where precariously small weight increases are sometimes regarded as significant. In some laboratories the gonadotropic potencies of the urines are expressed in terms of units per 24 hours, in others they are calculated as units per liter. Such differences in methodology and in expression of the data derived from the assays, in addition to the day-to-day variation in excretion rate of the subjects studied, render the values currently available of low reliability.

It is agreed that a standard of reference for urinary hormones would greatly facilitate the interpretation of the bioassays. There is, however, no single substance which can be chosen as a standard of reference. Several investigators have suggested that postmenopausal urine be con-

concentrated and used as a standard (1, 75, 104, 162). This suggestion has been made chiefly because this gonadotropic substance is present in urine in larger amounts than other "secreted" hormones. Its choice is also based on the inadequately established assumption that this gonadotropin is of pituitary origin, constant in quality, and similar to other human secretion products (aside from those found in pregnancy and in certain tumors).

Certain generalizations can probably be stated, however, on the basis of our present knowledge, regarding the occurrence and properties of gonadotropins in body fluids of nonpregnant individuals (Table VII). The amount of hormone present in human urine before puberty is at or below the limits of the methods for concentration and assay now commonly in use. Women during reproductive life secrete barely detectable amounts of gonadotropins during the early and late parts of the menstrual period. It is fairly certain that there is an increase in titer in mid-cycle, excretions as high as 40-50 mouse uterine units (MUU) per 24 hr. having been reported. A close correspondence is claimed between the increased titer at mid-cycle, as determined by the hyperemia test, and the time of ovulation.⁵ Postmenopausal women secrete more than the maximum found in normal cyclic women, values as high as 100 MUU/24 hr. being reported. The concentration of gonadotropin present in urine of normal adult men is on the average higher than in women, being of the order of that found in women during mid-menstrual cycle peak. Much interest has centered on clinical use of such urinary gonadotropic assays, particularly in attempts to differentiate between gonadal hypofunction due secondarily to defects in the pituitary or attributable directly to defective gonads. Low urinary gonadotropic titers, of the order of those found in children, are indicative of pituitary deficiency, while higher titers, comparable to those found in the castrate, indicate gonadal failure.

XIII. RELATION OF PITUITARY GONADOTROPINS TO THOSE IN BODY FLUIDS

The gonadotropins in body fluids fall into two categories, those of pregnancy, derived from the chorion or uterus, and those of nonpregnant individuals, presumably of pituitary origin. As already mentioned, the urinary and serum hormones of nonpregnant individuals have never been purified and compared chemically with pituitary hormones. It is not known whether the physiological effects (follicle stimulation, inter-

⁵ Farris (60) reported that among 50 women, in whom donor insemination was made, the maximum number of the conceptions (60%) occurred between days 11 and 13 of the menstrual cycle, and a positive hyperemia test was found between these days in 54%.

stitial cell stimulation, and corpus luteum formation) of these urinary gonadotropes are due to a single hormone with multiple properties or to two hormones. Furthermore, it is not known whether the gonadotropins obtained from the body fluids of normal men, young women, postmenopausal women, or of castrates represent a single hormone or several hormonal variants.

In the absence of chemical characterization, several types of biological evidence have been used in efforts to determine the distinctive properties and interrelations of the hormones in body fluids. Studies have been made of the rate of excretion and length of survival in the circulation; of the ratio of the follicle-stimulating and interstitial cell-stimulating properties, in comparison with the gonadotropins present in the pituitary under the same physiological conditions; and of the ability of the gonadotropin to stimulate the pituitary.

A. Survival of Gonadotropins in the Circulation (Half-Life)

Observations on time of survival in the circulation of injected gonadotropins, coupled with assays of the gonadotropic content of the urine, give promise of a better understanding of the hormones in the pituitary and in body fluids. The length of time that injected gonadotropins remain in circulation has not as yet been determined with accuracy. Hypophyseal hormone (flavianates from pig pituitary) injected into rats or into rhesus monkeys was readily recovered from the urine as was human chorionic gonadotropin but equine serum gonadotropin did not pass the urinary filter (54). After injection of horse pituitary gonadotropin into rabbits, the concentration of the hormone in the rabbits' plasma was found to have been reduced 50% by 6 hours; hence a 6-hour half-life by this test (178). The half-life of PMS has been found to be much longer; when injected into the gelding it has been determined to be 144 hours; in mares, 244 hours; in a heterologous species, the rabbit, the period was somewhat shorter, 24-26 hours. The half-life of the hormone from blood and urine of pregnant women (HCG) appears to be relatively short; after injection into rats the concentration in plasma was reduced to one-half within 30 minutes; in the dog and horse, within 2-3 hours; in rabbits, in less than 6 hours; and in the human, in less than 24 hours (178). The gonadotropin in blood plasma of castrates and postmenopausal women also appears in their urine; nothing is known, however, regarding the half-life. Studies such as those reported above have demonstrated great differences in ability of the hormones to pass the urinary filter, also differences in the time of survival in the circulation, but the evidence available at present is

inadequate to afford a satisfactory basis for determination of the relationship between the hormones extracted from the pituitary and those found in body fluids of nonpregnant individuals.

B. Ratio of Follicle-Stimulating and Interstitial Cell-Stimulating Potency in the Pituitary and Body Fluids

Table VIII shows the proportion of follicle-stimulating and interstitial cell-stimulating potencies in pituitaries of several species, and the corresponding properties of the gonadotropins in the body fluid when they are known. It will be noted that the relative potency in FSH and ICSH varies considerably in pituitaries of different species. The ratio of FSH to ICSH in human pituitary (7, 8, 9, 171, 172) is as high, or higher, than in pituitaries commonly used for chemical fractionation (beef, sheep, ox). The pituitary of the rat is intermediate in this regard, which may be indicative of the physiological requirements of this commonly used assay animal.

The values listed in the table give no indication of the rate of secretion from the pituitaries. The differing ratios in the pituitary gonadotropic components are also not necessarily indicative of the total amounts of hormone present, though bioassays appear to show that the total quantity of gonadotropin is greater in pituitaries where the ratio of FSH is higher. The potency of pituitaries is actually the resultant of the combined action of the two gonadotropins, FSH and ICSH, and an increase in potency may be due either to an increase in amount of FSH, or to a more favorable proportion of FSH to ICSH. An interpretation of the increased gonadal response cannot therefore be made unless the minimal effective dose for each component is determined.

The importance of determining ratios of FSH to ICSH in pituitaries, or ratios of FSH-like to ICSH-like substances in body fluids, is illustrated in the evaluation of gonadotropic potencies after castration. The outstanding biological response to injection of castrate pituitary is follicular development, although at higher doses corpora lutea are occasionally formed. By such a criterion the follicle-stimulating potency would appear to be differentially increased in pituitaries of postmenopausal women and in human castrates of either sex. The gonadotropic content of the plasma and urine is also increased when gonads are absent; this gonadotropin is described as predominantly, or entirely, follicle-stimulating in character. This increase in gonadotropic potency, particularly in follicle-stimulating capacity following castration, appears to characterize other species as well as man. Gonadotropic activity in

TABLE VIII

PROPORTIONS OF FSH AND ICSH POTENCIES IN GONADOTROPINS EXTRACTED FROM THE PITUITARY COMPARED WITH THOSE FOUND IN BODY FLUIDS^a
(7, 8, 9, 17, 37, 49, 52, 58, 68, 138, 143)

Species	Anterior pituitary Ratio of potency FSH to ICSH	Body fluids		Ratio hypophy- seal to normal MED ^b
		Ratio of FSH like to ICSH-like activity		
		Urine	Plasma	
Purified hormones from sheep pituitary				
FSH	40 1			
ICSH	1 300			
Human				
Children	1 1			
Women, pregnant	(almost absent)	1 6,000-10,000		2-4
Women, nonpregnant	1 1	1 1-2 1		
Women, menopausal	3 1-10 1	5 1-6 1		
Men, adult	1 1-2 1	1 1-3 1		4
Monkey				
Adult female				
cycle day 1	1 2-1 4			
9	1 2.5-1 3			
11	1 10			
15	1 10			
22	1 3			
Adult male	1 3			
Rat				
Adult female	1 2.5-1 3			
Adult male	1 1-1 2			
Castrate female	1 2-1 3			
Castrate male	1 1			
Pituitary 10% alcohol extract				
Whale	≥ 4 1			
Pig	1 2.5			
Sheep	1 2.5-1 5			
Beef	1 4			
Horse, nonpregnant	1 1		1 5	2
Horse, pregnant			1 2-1 3	2-4

^a Assayed in hypophysectomized immature female rats

^b Multiple of minimal effective dose in normal immature female rats required for interstitial tissue repair in hypophysectomized female rats

the pituitary and blood plasma is increased in both male and female rats after castration, and the predominant reaction is again follicle stimulation (37, 38, 40, 111, 124, 125). However, on analysis of the ratio of FSH to ICSH in pituitaries of male castrates by assay in hypophysectomized rats, it has been found that both the follicle-stimulating and interstitial cell-stimulating capacities are increased, although not to the same degree.⁶ Interstitial cell stimulation remains the biological effect given by pituitary extracts from castrate rats at the lowest doses, just as in the normal rat.

Any further information available for comparisons of ratios of follicle-stimulating to interstitial cell-stimulating potencies in pituitaries and body fluids is very limited and does not constitute a sufficiently substantial basis for determining the relation between the pituitary hormones and the secreted hormone in body fluids. During pregnancy, the gonadotropic potency of human pituitary is very low and the gonadotropin content of blood and urine, other than that attributed to chorionic origin, is low, if present at all. Not much is known regarding the relative potency of FSH and ICSH in pituitaries of normal adult men and women. The proportion of FSH-like and ICSH-like substances in urine is reported to be similar in the two sexes; the interstitial cell-stimulating potency may be relatively higher in women (Table VIII). In young women there is a mid-cycle increase in gonadotropic potency of the urine which has commonly been attributed to an increase in follicle-stimulating potency, but an increase in excretion of interstitial cell-stimulating substance at the time of ovulation has also been claimed (159). No adequate assays of human pituitaries at different times in the menstrual cycle are available for comparison with the urinary content. Data from another primate, the rhesus monkey, are, however, pertinent. The pituitary of the monkey has been reported to contain an increased content of gonadotropic potency (both FSH and ICSH) between the 9th and 11th days of the 28-day menstrual cycle. Between the 11th and 15th days of the cycle the total gonadotropic content was found to have decreased, and ICSH had increased relative to FSH (143). Ovulation in the monkey, as in the human female, is assumed to occur midway in the cycle.

⁶ In the male rat pituitary the follicle-stimulating potency is increased 2- to 4-fold, that of the interstitial cell stimulating potency 2-fold, so that the ratio of FSH:ICSH is changed from 1:2 to between 1:1 and 1:2 (37, 38). Though the blood plasma of castrates reestablished follicular growth when injected into hypophysectomized rats, no evidence has been presented for repair of interstitial cells on administration of plasma from normal or castrate rats in daily doses as high as 6-8 cc (40).

C. Pituitary Stimulation by the Various Gonadotropins

There are differences between pituitary gonadotropins and the various circulating hormones with respect to their potency in normal immature rats and in rats deprived of the pituitary. The potency of equine gonadotropin is greater in normal than in hypophysectomized rats; the minimal effective dose in hypophysectomized rats being four times greater. At a given dose level the ovaries of hypophysectomized rats are not as large, follicular development is not as elaborate, and corpora lutea form less frequently. Similarly, four times as much human chorionic gonadotropin is required to produce the minimal gonadotropic effect in hypophysectomized rats as is required for ovarian stimulation in the normal rat. Equine gonadotropin and human chorionic gonadotropin, when injected into hypophysectomized rats at the minimal effective levels, show the same end point, namely, repair of the interstitial tissue. Equine gonadotropin is unlike human chorionic gonadotropin in that it is an effective stimulant to follicular growth in hypophysectomized rats at doses only slightly above minimal, whereas chorionic gonadotropin stimulates follicles in hypophysectomized rats only at excessive doses.

The gonadotropic principles in the serum of nonpregnant as well as pregnant mares, in urine of normal adult men, and in blood and urine of pregnant women have the property in common of being effective ovarian stimulants in normal immature rats at a fraction (usually one-half to one-fourth) the dose necessary to repair the ovarian interstitial tissue in the hypophysectomized rat (68). The greater effectiveness in normal rats is attributed to the capacity of these substances to stimulate the pituitary to secrete gonadotropins. The secreted hormone then acts synergistically with the injected gonadotropin. Inasmuch as these body fluid gonadotropins vary greatly in their follicle-stimulating capacities, the ability to stimulate the pituitary is attributed to their more basic property, the interstitial cell-stimulating capacity. Although human chorionic gonadotropin, a predominantly interstitial cell-stimulating hormone, is the most effective among the circulating gonadotropins as a pituitary stimulant, hypophyscal ICSH, which otherwise resembles most closely the human chorionic gonadotropin in biological effects, exhibits little or no ability to stimulate the pituitary. Pituitary ICSH manifests only slight gonadotropic activity in immature normal female rats; even when tested at ten times the dosage necessary to show its specific action in hypophysectomized rats, it elicits little or no gonadotropic activity in normal immature rats. This capacity to stimulate the anterior pituitary, which characterizes many of the gonadotropins in

body fluids, does not therefore assist in determining their relationship to the pituitary gonadotropins.

ACKNOWLEDGMENTS

I wish to express my appreciation to the staff of the Institute of Experimental Biology for the assistance afforded in preparation of this chapter, particularly to Dr. F. Carter, who has assisted in all phases of its preparation, to Dr. M. C. Woods for chemical evaluations, and to Dr. M. M. Nelson for assistance in dealing with the vitamin-hormonal relationships in reproduction.

REFERENCES

1. Albert, A., *Proc. Staff Meetings Mayo Clinic* **31**, 341 (1956).
2. Albert, A., and Berkson, J., *J. Clin. Endocrinol. and Metabolism* **11**, 805 (1951).
3. Aschheim, S., and Zondek, B., *Klin. Wochschr.* **7**, 8 (1928).
4. Astwood, E. B., *Endocrinology* **23**, 309 (1941).
5. Astwood, E. B., and Greep, R. O., *Proc. Soc. Exptl. Biol. Med.* **38**, 713 (1938).
6. Austin, C. R., *J. Endocrinol.* **6**, 293 (1950).
7. Bahn, R. C., Lorenz, N., Bennett, W. A., and Albert, A., *Endocrinology* **52**, 135 (1953).
8. Bahn, R. C., Lorenz, N., Bennett, W. A., and Albert, A., *Proc. Soc. Exptl. Biol. Med.* **82**, 777 (1953).
9. Bahn, R. C., Lorenz, N., Bennett, W. A., and Albert, A., *Endocrinology* **53**, 455 (1953).
10. Bergenstal, D. M., 1953, see (95).
11. Biskind, M. S., *Vitamins and Hormones* **4**, 147 (1946).
12. Blandau, R. J., *Fertility and Sterility* **6**, 391 (1955).
13. Breneman, W. R., and Mason, R. C., *Endocrinology* **48**, 752 (1951).
14. Bromberg, Y. M., Brzezinski, A., Rozin, S., and Sulman, F. C., *Acta Endocrinol.* **7**, 31 (1951).
15. Brown, W. E., and Bradbury, J. T., *Am. J. Obstet. Gynecol.* **53**, 749 (1947).
16. Browne, J. S. L., and Venning, E. M. H., *Am. J. Physiol.* **123**, 26 (1938).
17. Bruner, J. A., *J. Clin. Endocrinol.* **11**, 360 (1951).
18. Bukovics, E., and Wohlzogen, F. X., *Acta Endocrinol.* **14**, 273 (1953).
19. Byrnes, W. W., and Meyer, R. K., *Endocrinology* **48**, 133 (1951).
20. Byrnes, W. W., and Meyer, R. K., *Endocrinology* **49**, 449 (1951).
21. Carter, F., Simpson, M. E., and Evans, H. M., *Anot. Record* **130**, 283 (1958).
22. Cartland, C. F., and Nelson, J. W., *J. Biol. Chem.* **119**, 59 (1937).
23. Chow, B. F., Van Dyke, H. B., Greep, R. O., Rothen, A., and Shedlovsky, T., *Endocrinology* **30**, 650 (1942).
24. *Ciba Colloquia Endocrinol.* **5** (1953).
25. Claesson, L., Hogberg, B., Rosenberg, T., and Westman, A., *Acta Endocrinol.* **7**, 1 (1948).
26. Clegg, M. T., Boda, J. M., and Cole, H. H., *Endocrinology* **54**, 448 (1954).
27. Coffin, D. L., Munson, T. O., and Scully, R. E., *J. Am. Vet. Med. Assoc.* **121**, 352 (1952).
28. Cole, H. H., *Acta Endocrinol.* **24**, Suppl. **31**, 108 (1957).
29. Cole, H. H., and Goss, H., in "Essays in Biology in Honor of Herbert M. Evans," p. 107. Univ. of Calif. Press, Berkeley, California, 1943.

- 30 Cole, H H, Goss, H, and Boda, J, *J Clin Endocrinol* 10, 432 (1950)
- 31 Cole, H H, Hamburger, C, and Niemann Sprensen, A, *Acta Endocrinol* 26, 286 (1957)
- 32 Cole, H H, and Hart, G H, *Am J Physiol* 93, 57 (1930)
- 33 Cole, H H, and Miller, R F, *Am J Physiol* 104, 165 (1933)
- 34 Cole, H H, and Saunders, P J, *Endocrinology* 19, 199 (1935)
- 35 Cole, R D, and Li, C H, *J Biol Chem* 213, 197 (1955)
- 36 Collip, J B, and Anderson, E M, *Lancet* 226, 76 (1934)
- 37 Contopoulos, A N, *Anat Record* 130, 288 (1958)
- 38 Contopoulos, A N, Simpson, M E, and Koneff, A A, *Endocrinology* (1958), in press
- 39 Cowie, A T, and Folley, S J, in "The Hormones" (G Pincus and K V Thumann, eds), Vol 3, p 309 Academic Press, New York, 1955
- 40 Cozens, D A, and Nelson, M M, *Proc Soc Exptl Biol Med* 98, 123 (1958)
- 41 Davis, M E, and Koff, A K, *Am J Obstet Gynecol* 36, 183 (1938)
- 42 Diczfalussy, E, *Acta Endocrinol* 12, Suppl 12, 7 (1953)
- 43 Diczfalussy, E, *Acta Endocrinol* 17, 58 (1954)
- 44 Drescher, J, and Stange, H H, *Acta Endocrinol* 19, 289 (1955)
- 45 du Vigneaud, V, Lawler, H C, and Popenoe, E A, *J Am Chem Soc* 75, 4880 (1953)
- 46 Dvoskin, S, *Am J Anat* 75, 289 (1944)
- 47 Ershoff, B H, *Vitamins and Hormones* 10, 79 (1952)
- 48 Evans, H M, and Bishop, K S, *Science* 56, 650 (1922)
- 49 Evans, H M, and Gorbman, A, *Proc Soc Exptl Biol Med* 49, 674 (1942)
- 50 Evans, H M, Kohls, C L, and Wonder, D H, *J Am Med Assoc* 108, 287 (1937)
- 51 Evans, H M, Meyer, K, and Simpson, M E, *Proc Soc Exptl Biol Med* 28, 845 (1931)
- 52 Evans, H M, and Simpson, M E, in "The Hormones," (G Pincus and K V Thumann, eds), Vol 2, p 352 Academic Press, New York, 1950
- 53 Evans, H M, Simpson, M E, and Austin, P R, *J Exptl Med* 58, 545 (1933)
- 54 Evans, H M, Simpson, M E, and Austin, P R, *J Exptl Med* 58, 561 (1933)
- 55 Evans, H M, Simpson, M E, and Lyons, W R, *Proc Soc Exptl Biol Med* 46, 586 (1941)
- 56 Evans, H M, Simpson, M E, Lyons, W R, and Turpeinen, K, *Endocrinology* 28, 933 (1941)
- 57 Evans, H M, Simpson, M E, and Pencharz, R I, *Cold Spring Harbor Symposia Quant Biol* 5, 229 (1937)
- 58 Evans, H M, Simpson, M E, Tolkdorf, S, and Jensen, H, *Endocrinology* 25, 529 (1939)
- 59 Everett, J W, *Endocrinology* 59, 560 (1950)
- 60 Harris, E J, *Am J Obstet Gynecol* 56 347 (1948)
- 61 Fernald, H L, in "Sex and Internal Secretions" (E Allen, ed), p 968 Williams & Wilkins, Baltimore, Maryland, 1939
- 62 Fernald, H L, Hiss, F. L, and Leonard, S L, *Am J Physiol* 97, 291 (1931)
- 63 Finerty, J C, and Meyer, R K, *Endocrinology* 46 494 (1950)
- 64 Folley, S J, Greenbaum, A L, and Roy, A, *J. Endocrinol* 6, 121 (1919).

65. Follis, R. H., "Pathology of Nutritional Disease." C. C Thomas, Springfield, Illinois, 1948.
66. Forbes, T. R., *Endocrinology* 49, 218 (1951).
67. Fowler, R. E., and Edwards, R. G., *J. Endocrinol.* 15, 374 (1957).
68. Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology* 27, 793 (1940).
69. Fraenkel-Conrat, H., Simpson, M. E., Li, C. H., and Evans, H. M., *Anales fac. med. Montevideo* 25, 627 (1940).
70. Fried, P. H., and Rakoff, A. E., *J. Clin. Endocrinol.* 10, 423 (1950).
71. Cey, C. O., Seegar, G. E., and Hellman, L. M., *Science* 88, 308 (1938).
72. Greenblatt, R. B., Clark, S. L., and West, R. M., *Fertility and Sterility* 1, 533 (1950).
73. Creep, R. O., and Jones, I. C., *Recent Progr. in Hormone Research* 5, 197 (1950).
74. Creep, R. O., Van Dyke, H. B., and Chow, B. F., *Endocrinology* 30, 635 (1942).
75. Hamburger, C., in "Hormone Assay" (C. W. Emmens, ed.), p. 174. Academic Press, New York, 1950.
76. Hamburger, C., *Acta Endocrinol.* 24, Suppl. 31, 59 (1957).
- 76a. Hamburger, C., and Johnsen, S. G., *Acta Endocrinol.* 26, 1 (1957).
77. Hamlett, G. W. D., *Am. J. Physiol.* 118, 664 (1937).
78. Hartman, C. G., *Bull. Johns Hopkins Hosp.* 63, 351 (1938).
79. Hays, E. E., and Steelman, S. L., in "The Hormones" (G. Pincus and K. V. Thimann, eds.), Vol. 3, p. 201. Academic Press, New York, 1955.
80. Heitman, H., Jr., and Cole, H. H., *J. Animal Sci.* 15, 970 (1956).
81. Hertz, R., *Vitamins and Hormones* 4, 135 (1946).
82. Hertz, R., *Recent Progr. in Hormone Research* 2, 161 (1948).
83. Hisaw, F. L., Creep, R. O., and Fevold, H. L., *Anat. Record* 61, Suppl. 24 (1935).
84. Hohlweg, W., *Klin. Wochschr.* 13, 92 (1934).
85. Knobil, E., Morse, A., and Creep, R. O., *Anat. Record* 124, 320 (1956).
86. Koneff, A. A., *Anat. Record* 74, 363 (1939).
87. Laufer, A., and Sulman, F. G., *J. Clin. Endocrinol. and Metabolism* 16, 1151 (1950).
88. *League Nations Bull. Health Organisation* 8, 684 (1939).
89. *League Nations Bull. Health Organisation* 8, 898 (1939).
90. *League Nations Bull. Health Organisation* 8, 909 (1939).
91. Leatham, J. H., and Levin, L., *Endocrinology* 29, 6 (1941).
92. Leonard, S. L., *Am. J. Physiol.* 98, 406 (1931).
93. Leonard, S. L., *Proc. Soc. Exptl. Biol. Med.* 30, 403 (1932).
94. Li, C. H., *J. Am. Chem. Soc.* 72, 2815 (1950).
95. Li, C. H., *Federation Proc.* 16, 775 (1957), see also reference (10).
96. Li, C. H., *Advances in Protein Chem.* 12, 269 (1957).
97. Li, C. H., in "Symposium on Protein Structure: International Union of Pure and Applied Chemistry, Paris, 1957" (A. Neuberger, ed.), p. 303. Methuen & Co., Ltd. London, 1958.
98. Li, C. H., and Evans, H. M., in "The Hormones" (G. Pincus and K. V. Thimann, eds.), Vol. 1, p. 631. Academic Press, New York, 1948.
99. Li, C. H., Evans, H. M., and Wonder, D. H., *J. Gen. Physiol.* 23, 733 (1940).
100. Li, C. H., and Pedersen, K. O., *J. Gen. Physiol.* 35, 629 (1952).

101. Li, C. H., Simpson, M. E., and Evans, H. M., *J. Am. Chem. Soc.* 64, 367 (1942).
102. Li, C. H., Simpson, M. E., and Evans, H. M., *Science* 109, 445 (1949).
103. Loraine, J. A., *Vitamins and Hormones* 14, 305 (1956).
104. Loraine, J. A., and Brown, J. B., *J. Clin. Endocrinol. and Metabolism* 16, 1160 (1956).
105. Ludwig, D. J., *Endocrinology* 46, 453 (1950).
106. Lyon, R. A., Simpson, M. E., and Evans, H. M., *Endocrinology* 53, 674 (1953).
107. Lyons, W. R., *Cold Spring Harbor Symposia Quant. Biol.* 5, 196 (1937).
108. Lyons, W. R., *Proc. Soc. Exptl. Biol. Med.* 54, 65 (1943).
109. Maddock, W. O., Leach, R. B., Tokuyama, I., Paulsen, C. A., Nelson, W. O., Jungck, E. C., and Heller, C. G., *Acta Endocrinol. Suppl.* 28, 55 (1956).
110. Marden, W. G. R., *Endocrinol.* 50, 456 (1952).
111. Martins, T., *Mem. inst. Oswaldo Cruz Suppls.* 1-12, 242 (1926-1929).
112. McArthur, J. W., *Endocrinology* 50, 304 (1952).
113. Mulinos, M. G., and Pomerantz, L., *J. Nutrition* 19, 493 (1940).
114. Nalbandov, A. V., *Fertility and Sterility* 3, 100 (1952).
115. Nalbandov, A. V., *Poultry Science* 32, 86 (1953).
116. Nelson, M. M., *Federation Proc.* 14, 446 (1955).
117. Nelson, M. M., *Pediatrics* 19, 764 (1957).
118. Nelson, M. M., and Evans, H. M., *Endocrinology* 55, 543 (1954).
119. Nelson, M. M., and Evans, H. M., *J. Nutrition* 55, 151 (1955).
120. Nelson, M. M., and Evans, H. M., *Proc. Soc. Exptl. Biol. Med.* 91, 614 (1956).
121. Nelson, M. M., Lyons, W. R., and Evans, H. M., *Endocrinology* 48, 726 (1951).
122. Nelson, W. O., *Acta Endocrinol. Suppl.* 28, 7 (1956).
123. Otsuka, H., and Noda, Y., *J. Biochem. (Tokyo)* 41, 547 (1954).
124. Paesi, F. J. A., and de Jongh, S. E., *Acta Endocrinol.* 15, 1 (1954).
125. Paesi, F. J. A., de Jongh, S. E., Hoogstra, M. J., and Engellbregt, A., *Acta Endocrinol.* 19, 49 (1955).
126. Papkoff, H., and Li, C. H., *J. Biol. Chem.* 231, 367 (1956).
127. Pencharz, R. I., and Long, J. A., *Science* 74, 206 (1931).
128. Philipp, E., *Zentr. Gynäkol.* 54, 1858 (1930).
129. Raacke, I. D., Li, C. H., and Lostroh, A. J., *Acta Endocrinol.* 17, 366 (1954).
130. Raacke, I. D., Lostroh, A. J., Boda, J. M., and Li, C. H., *Acta Endocrinol.* 26, 377 (1957).
131. Ray, E. W., Averill, S. C., Lyons, W. R., and Johnson, R. E., *Endocrinology* 56, 359 (1955).
132. Rimington, C., and Rowlands, I. W., *Biochem. J.* 38, 54 (1944).
133. Robinson, T. J., *J. Agr. Sci.* 41, 6 (1951).
134. Robinson, T. J., *J. Endocrinol.* 10, 117 (1954).
135. Rowson, L. E., *J. Endocrinol.* 7, 260 (1951).
136. Salhanick, H. A., Hisaw, F. L., and Zarrow, M. X., *J. Clin. Endocrinol. and Metabolism* 12, 310 (1952).
137. Schneider, W. G., and Frahm, H., *Acta Endocrinol.* 20, 279 (1955).
138. Simpson, M. E., *Western J. Surg. Obstet. Gynecol.* 52, 287 (1944).
139. Simpson, M. E., and Evans, H. M., *Endocrinology* 39, 281 (1946).
140. Simpson, M. E., Li, C. H., and Evans, H. M., *Endocrinology* 30, 977 (1942).
141. Simpson, M. E., Li, C. H., and Evans, H. M., *Endocrinology* 35, 96 (1944).
142. Simpson, M. E., Li, C. H., and Evans, H. M., *Endocrinology* 48, 370 (1951).

65. Follis, R. H., "Pathology of Nutritional Disease." C. C Thomas, Springfield, Illinois, 1946.
66. Forbes, T. R., *Endocrinology* 49, 218 (1951).
67. Fowler, R. E., and Edwards, R. C., *J. Endocrinol.* 15, 374 (1957).
68. Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology* 27, 793 (1940).
69. Fraenkel-Conrat, H., Simpson, M. E., Li, C. H., and Evans, H. M., *Anales fac. med. Montevideo* 25, 627 (1940).
70. Fried, P. H., and Rakoff, A. E., *J. Clin. Endocrinol.* 10, 423 (1950).
71. Gey, G. O., Seegar, G. E., and Hellman, L. M., *Science* 88, 306 (1936).
72. Greenblatt, R. B., Clark, S. L., and West, R. M., *Fertility and Sterility* 1, 533 (1950).
73. Greep, R. O., and Jones, I. C., *Recent Progr. in Hormone Research* 5, 197 (1950).
74. Greep, R. O., Van Dyke, H. B., and Chow, B. F., *Endocrinology* 30, 635 (1942).
75. Hamburger, C., in "Hormone Assay" (G. W. Emmens, ed.), p. 174. Academic Press, New York, 1950.
76. Hamburger, C., *Acta Endocrinol.* 24, Suppl. 31, 59 (1957).
- 76a. Hamburger, C., and Johnsen, S. C., *Acta Endocrinol.* 26, 1 (1957).
77. Hamlett, G. W. D., *Am. J. Physiol.* 118, 684 (1937).
78. Hartman, G. C., *Bull. Johns Hopkins Hosp.* 63, 351 (1936).
79. Hays, E. E., and Steelman, S. L., in "The Hormones" (G. Pincus and K. V. Thimann, eds.), Vol. 3, p. 201. Academic Press, New York, 1955.
80. Heitman, H., Jr., and Cole, H. H., *J. Animal Sci.* 15, 970 (1956).
81. Hertz, R., *Vitamins and Hormones* 4, 135 (1946).
82. Hertz, R., *Recent Progr. in Hormone Research* 2, 161 (1946).
83. Hisaw, F. L., Greep, R. O., and Fevold, H. L., *Anat. Record* 61, Suppl. 24 (1935).
84. Hohlweg, W., *Klin. Wochschr.* 13, 92 (1934).
85. Knobil, E., Morse, A., and Greep, R. O., *Anat. Record* 124, 320 (1956).
86. Koneff, A. A., *Anat. Record* 74, 383 (1939).
87. Laufer, A., and Sulman, F. G., *J. Clin. Endocrinol. and Metabolism* 16, 1151 (1956).
88. *League Nations Bull. Health Organisation* 8, 884 (1939).
89. *League Nations Bull. Health Organisation* 8, 898 (1939).
90. *League Nations Bull. Health Organisation* 8, 909 (1939).
91. Leatham, J. H., and Levin, L., *Endocrinology* 29, 8 (1941).
92. Leonard, S. L., *Am. J. Physiol.* 98, 406 (1931).
93. Leonard, S. L., *Proc. Soc. Exptl. Biol. Med.* 30, 403 (1932).
94. Li, C. H., *J. Am. Chem. Soc.* 72, 2815 (1950).
95. Li, C. H., *Federation Proc.* 16, 775 (1957), see also reference (19).
96. Li, C. H., *Advances in Protein Chem.* 12, 269 (1957).
97. Li, C. H., in "Symposium on Protein Structure: International Union of Pure and Applied Chemistry, Paris, 1957" (A. Neuberger, ed.), p. 303. Methuen & Co., Ltd. London, 1958.
98. Li, C. H., and Evans, H. M., in "The Hormones" (G. Pincus and K. V. Thimann, eds.), Vol. 1, p. 631. Academic Press, New York, 1948.
99. Li, C. H., Evans, H. M., and Wonder, D. H., *J. Gen. Physiol.* 23, 733 (1940).
100. Li, C. H., and Pedersen, K. O., *J. Gen. Physiol.* 33, 629 (1952).

- 101 Li, C H, Simpson, M E, and Evans, H M, *J Am Chem Soc* 64, 367 (1942)
- 102 Li, C H, Simpson, M E, and Evans, H M, *Science* 109, 445 (1949)
- 103 Loraine, J A, *Vitamins and Hormones* 14, 305 (1956)
- 104 Loraine, J A, and Brown, J B, *J Clin Endocrinol and Metabolism* 16, 1180 (1956)
- 105 Ludwig, D J, *Endocrinology* 45, 453 (1950)
- 106 Lyon, R A, Simpson, M E, and Evans, H M, *Endocrinology* 53, 674 (1953)
- 107 Lyons, W R, *Cold Spring Harbor Symposia Quant Biol* 5, 198 (1937)
- 108 Lyons, W R, *Proc Soc Exptl Biol Med* 54, 65 (1943)
- 109 Maddock, W O, Leach, R B, Tokuyama, I, Paulsen, C A, Nelson, W O, Jungck, E C, and Heller, C C, *Acta Endocrinol Suppl* 28, 55 (1956)
- 110 Marden, W G R, *Endocrinol* 50, 456 (1952)
- 111 Martins, T, *Mem Inst Oswaldo Cruz Suppl* 1-12, 242 (1928-1929)
- 112 McArthur, J W, *Endocrinology* 50, 304 (1952)
- 113 Mulinos, M G, and Pomerantz, L, *J Nutrition* 19, 493 (1940)
- 114 Nalbandov, A V, *Fertility and Sterility* 3, 100 (1952)
- 115 Nalbandov, A V, *Poultry Science* 32, 88 (1953)
- 116 Nelson, M M, *Federation Proc* 14, 446 (1955)
- 117 Nelson, M M, *Pediatrics* 19, 764 (1957)
- 118 Nelson, M M, and Evans, H M, *Endocrinology* 55, 543 (1954)
- 119 Nelson, M M, and Evans, H M, *J Nutrition* 55, 151 (1955)
- 120 Nelson, M M, and Evans, H M, *Proc Soc Exptl Biol Med* 91, 614 (1956)
- 121 Nelson, M M, Lyons, W R, and Evans, H M, *Endocrinology* 48, 726 (1951)
- 122 Nelson, W O, *Acta Endocrinol Suppl* 28, 7 (1956)
- 123 Otsuka, H, and Noda, Y, *J Biochem (Tokyo)* 41, 547 (1954)
- 124 Paesi, F J A, and de Jongh, S E, *Acta Endocrinol* 15, 1 (1954)
- 125 Paesi, F J A, de Jongh, S E, Hoogstra, M J, and Engelbregt, A, *Acta Endocrinol* 19, 49 (1955)
- 126 Papkoff, H, and Li, C H, *J Biol Chem* 231, 367 (1958)
- 127 Pencharz, R I, and Long, J A, *Science* 74, 206 (1931)
- 128 Philipp, E, *Zentr Gynakol* 54, 1858 (1930)
- 129 Raacke, I D, Li, C H, and Lostroh, A J, *Acta Endocrinol* 17, 366 (1954)
- 130 Raacke, I D, Lostroh, A J, Boda, J M, and Li, C H, *Acta Endocrinol* 26, 377 (1957)
- 131 Ray, E W, Averill, S C, Lyons, W R, and Johnson, R E, *Endocrinology* 56, 350 (1955)
- 132 Rimington, C, and Rowlands, I W, *Biochem J* 38, 54 (1944)
- 133 Robinson, T. J, *J Agr Sci* 41, 6 (1951)
- 134 Robinson, T. J, *J Endocrinol* 10, 117 (1954)
- 135 Rowson, L E, *J Endocrinol* 7, 260 (1951)
- 136 Salhanick, H A, Hisaw, F L, and Zarrow, M A, *J Clin Endocrinol and Metabolism* 12, 310 (1952)
- 137 Schneider, W G, and Frahm, H, *Acta Endocrinol* 20, 279 (1955)
- 138 Simpson, M E, *Western J Surg Obstet Gynecol* 52, 287 (1944)
- 139 Simpson, M E, and Evans, H M, *Endocrinology* 39, 281 (1940)
- 140 Simpson, M E, Li, C H, and Evans, H M, *Endocrinology* 30, 977 (1942)
- 141 Simpson, M F, Li, C H, and Evans, H M, *Endocrinology* 35, 96 (1944)
- 142 Simpson, M E, Li, C H, and Evans, H M, *Endocrinology* 48, 370 (1951)

143. Simpson, M. E., van Wagenen, G., and Carter, F., *Proc. Soc. Exptl. Biol. Med.* 91, 6 (1956).
144. Smith, P. E., *J. Am. Med. Assoc.* 88, 156 (1927).
145. Smith, P. E., *Am. J. Physiol.* 60, 114 (1927).
146. Smith, P. E., *Am. J. Anat.* 45, 205 (1930).
147. Smith, P. E., in "Les Hormones Sexuelles" (S. Brouha, ed.), p. 291. Hermann, Paris, 1938.
148. Smith, P. E., *Yale J. Biol. and Med.* 17, 281 (1914).
149. Smith, P. E., *Endocrinology* 55, 655 (1954).
150. Squire, P. G., and Li, C. H., *Science* 127, 32 (1958).
151. Srebnik, H. H., and Nelson, M. M., *Anat. Record* 127, 372 (1957).
152. Steelman, S. L., Kelly, T. L., Segaloff, A., and Weber, G. F., *Endocrinology* 59, 256 (1956).
153. Steelman, S. L., Lamont, W. A., and Baltes, B. J., *Endocrinology* 55, 216 (1955).
154. Steelman, S. L., Lamont, W. A., and Baltes, B. J., *Acta Endocrinol.* 22, 186 (1956).
155. Steelman, L. S., and Pohley, F. M., *Endocrinology* 53, 604 (1953).
156. Sturgis, S. H., *Fertility and Sterility* 1, 40 (1950).
157. Sturgis, S. H., and Haour, P., *Fertility and Sterility* 2, 347 (1951).
158. Talbert, G. B., di Pillo, F., and Gordis, L., *Endocrinology* 61, 611 (1957).
159. Taymor, M. L., and Sturgis, S. H., *Fertility and Sterility*, in press.
160. Umbaugh, R. E., *Fertility and Sterility* 2, 243 (1951).
161. Van Dyke, H. B., Pan, S. Y., and Shedlovsky, T., *Endocrinology* 45, 563 (1950).
162. van Gilse, H. A., Nass, G. A. G., and Kassenaar, A. A. H., *Acta Endocrinol.* 24, 91 (1957).
163. van Wagenen, G., *Anat. Record* 29, 396 (1925).
164. van Wagenen, G., and Simpson, M. E., *Proc. Soc. Exptl. Biol. Med.* 90, 346 (1955).
165. van Wagenen, G., and Simpson, M. E., *Endocrinology* 51, 316 (1957).
166. van Wagenen, G., and Simpson, M. E., *Rev. suisse zool.* 64, 807 (1957).
167. Walsh, E. L., Guyler, W. K., and McCullagh, D. R., *Am. J. Physiol.* 107, 508 (1934).
168. White, A., Bonsnes, R. W., and Long, C. N. H., *J. Biol. Chem.* 143, 447 (1942).
169. Wilhelmi, A. E., in "The Hypophyseal Growth Hormone, Nature and Actions" (R. W. Smith, Jr., O. H. Gaebler, and C. N. H. Long, eds.), p. 59. Blakiston Div., McGraw-Hill, New York, 1954.
170. Williams, P. C., *Nature* 145, 388 (1940).
171. Witschi, E., *Endocrinology* 27, 437 (1940).
172. Witschi, E., and Riley, G. M., *Endocrinology* 28, 565 (1940).
173. Woods, M. C., and Simpson, M. E., unpublished.
174. Wooten, E., Nelson, M. M., Simpson, M. E., and Evans, H. M., *Endocrinology* 56, 59 (1955).
175. Zarrow, M. X., and Neber, G. M., *Endocrinology* 56, 1 (1955).
176. Zondek, B., and Aschheim, S., *Klin. Wochschr.* 6, 248 (1927).
177. Zondek, B., and Sulman, F. G., "The Antigonadotropic Factor." Williams & Wilkins, Baltimore, Maryland, 1942.
178. Zondek, B., and Sulman, F., *Vitamins and Hormones* 3, 297 (1945).

CHAPTER 4

Role of Gonadal Hormones in Reproductive Processes

C W EMMENS

	<i>Page</i>
I Introduction	112
A Types of Hormone Concerned	112
B Sources of Steroid Hormones	114
C General Actions, Blood Levels, and Dosage	115
1 Androgens	115
2 Estrogens	116
3 Progesterone	118
II Androgens	119
A Normal Role at Puberty and the Breeding Season	119
B Effects of Castration	120
C Effects on the Male Reproductive Organs	122
1 External Genitalia	122
2 Internal Genitalia	123
D Effects on the Female	125
E Effects on the Embryo	127
F Assay	127
1 Biological Assay	127
2 Chemical Assay	128
III Estrogens	129
A Normal Role at Puberty and the Breeding Season	129
B Effects of Ovariectomy	129
C Effects on the Female Reproductive Organs	130
D Effects on the Male	133
E Effects on the Embryo	134
F Assay	135
1 Biological Assay	135
2 Chemical Assay	136
IV Progesterone	137
A Normal Role in the Nonpregnant Female	137
B Normal Role in the Pregnant Female	138
C Assay	139
1 Biological Assay	139
2 Chemical Assay	140
V The Estrous Cycle	140
A General Considerations	140
B Cyclic Changes in the Female Reproductive Organs	142
C Factors Affecting the Type of Estrous Cycle	144
VI Reliun	146
A Nature and Distribution	146
B Physiological Action	146
C Assay of Reliun	147
References	147

I. INTRODUCTION

A. *Types of Hormone Concerned*

With the exception of relaxin, all of the hormones with which we are concerned in this chapter are steroids. They are related to cholesterol, although not necessarily derived from it biosynthetically, and are part of a wide range of compounds formed in the gonads, the adrenal cortex, and the placenta.

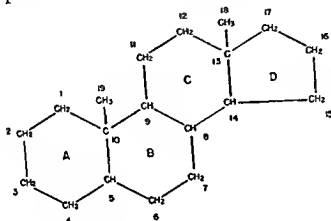


FIG. 1. Details of the steroid nucleus showing nomenclature of the rings and carbon atoms.

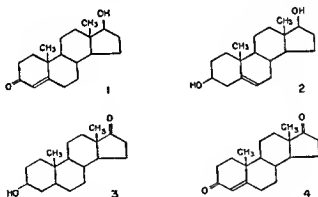


FIG. 2. Four important androgens: (1) testosterone; (2) androstenediol; (3) androsterone; (4) androstenedione.

The basic steroid nucleus, the cyclopentanoperhydrophenanthrene nucleus, is shown in Fig. 1. Compounds with actions resembling male hormone, the androgens, are characterized by having a carbon atom in each of positions 18 and 19, and may be typified by testosterone, the most potent natural androgen. This and other important androgens are shown in Fig. 2. Compounds which induce cornification of the rodent

vagina, and which otherwise have actions resembling female hormone, the estrogens, are characterized by having a carbon atom in position 18 but not in position 19. These are typified by 17β estradiol, probably the most potent of the natural estrogens, shown together with some other natural estrogens in Fig 3. It is also characteristic of the natural estrogens that they have an unsaturated A-ring. Progestational steroids with actions resembling progesterone are very few in number and are typified by that compound (Fig 4) and by having a structure like testosterone with an additional side chain in position 17. Adrenocortical compounds

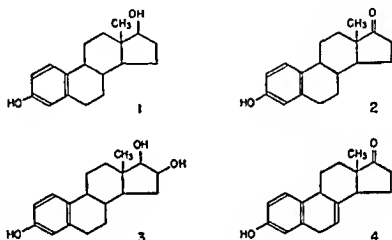


FIG 3 Four important natural estrogens (1) estradiol (2) estrone, (3) estriol, (4) equilin

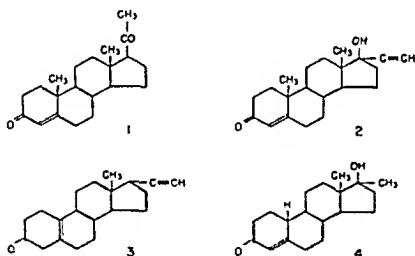


FIG 4 Progestogens (1) progesterone (2) ethynyltestosterone, (3) 17-ethinyl-5(10)-estracneolone (4) 17-ethyl-19-nor testosterone

are even more numerous than those listed above, in fact, the adrenal cortex manufactures practically the whole biological range, but those more characteristic of its action are not the subject of this chapter.

Detailed discussion of the interrelationships of steroids, of isomeric forms and methods of preparation may be sought in references (201) and (63).

B. Sources of Steroid Hormones

Androgens are found in the gonads of both sexes, the adrenal cortex, and probably the placenta [for a summary of references see (61)]. Testosterone has been found in the testes of many species, and in the spermatic vein, but is probably not formed by the adrenal gland. Its biosynthesis is at least in part controlled by the pituitary gonadotropic hormones and in some species it is very possibly the only testicular male hormone. However, Δ^4 -androstene-3,17-dione has also been isolated from both testes and the spermatic vein blood. Five androgens are apparently produced by the adrenal cortex; Δ^4 -androstene-3,17-dione; androstosterone; 11β -hydroxy- Δ^4 -androstene-3,17-dione, 11β -hydroxyepiandrosterone; and dehydroepiandrosterone (61).

Testosterone and Δ^4 -androstene-3,17-dione are formed from C^{14} -labeled acetate (19) in the testes of various species, probably without the formation of cholesterol as an intermediate. The steps in biosynthesis are now thought to be oxidation of pregnenolone, followed by hydroxylation to 17α -hydroxy progesterone and then to Δ^4 -androstene-3,17-dione. This last compound would form testosterone on reduction of the 17-ketone group.

Estrogens have been isolated from the gonads, adrenals, and placenta; the testes of the male may produce estrogen in large amounts, as, for instance, in the stallion. 17β -Estradiol has been isolated from all of these sources. Estrogen production in both sexes is stimulated by gonadotropins, particularly by pituitary luteinizing hormone and chorionic gonadotropin. Studies with C^{14} -labeled acetate (121) and labeled cholesterol have shown the production of estrone from acetate but not from cholesterol, and the production of equilin and equilinin (estrogens peculiar to the equidae) from sources other than estrone. It is thought that estrogen biosynthesis represents a rather special case in the general scheme of steroid synthesis, the origin of the aromatic ring A being still obscure. For detailed discussion Dorfman and Shipley (63) and Heard *et al.* (120) may be consulted.

Progesterone, the only natural substance of importance in its group, is produced by the corpus luteum, the placenta, the adrenal cortex, and most probably by the Graafian follicle even prior to ovulation. Its production in the corpus luteum of at least some species (outstandingly the rat) would seem to depend on prolactin from the anterior pituitary gland. In other species, notably the rabbit (216, 217), the luteotropic hormone may be estrogen. It has been assumed, mostly on indirect grounds, that the domestic animals and man resemble the rat rather than the rabbit in this regard. The stimulant for progesterone synthesis

in the adrenal gland seems to be adrenocorticotropin. Progesterone can be formed directly from cholesterol by the placenta (16) and is thought to be so formed in the endocrine glands proper; as indicated above, it is thought to be formed during the biosynthesis of testosterone.

C. General Actions, Blood Levels, and Dosage

Brief consideration will be given to some actions of the gonadal hormones other than those more specific to the reproductive tract. In addition to being stimulants of growth of the accessory reproductive organs and of sex receptivity, the gonadal steroids have various systemic actions of importance to an understanding of their over-all effects in the organism.

1. Androgens

Androgens cause nitrogen retention, but the nitrogen is promptly lost on cessation of administration. This is a specific protein anabolic effect [(145, 200) for summary]. A main effect is on skeletal muscle and occurs in the absence of the testes and various other endocrine glands. A large range of androgens has recently been examined in this regard by Kochakian and Tillotson (146). Increased sebaceous gland development and secretion are seen on androgen administration to both rats (65) and man (109). In the male, androgen elicits the characteristic behavior patterns of that sex in each particular species (10), but tends to be more important in the higher vertebrates in this regard. However, the clasping reflex of frogs and toads (193), and the male call (15) depend on androgen; male sexual behavior in reptiles of various species is lost on castration and restored by androgen injections.

The testes and the pituitary gland affect each other, the influence of the testis on the pituitary being exerted via the production of androgen. This apparently keeps the gonadotropin output of the pituitary gland within normal limits in the intact animal, excess of gonadotropin being formed on castration in the rat (169, 170). A similar relationship is seen in humans by measurement of urinary gonadotropin output (37). In birds, excess of androgen will inhibit the pituitary output of other hormones (196).

Blood levels of steroids have been reviewed recently by Tamm (236), although this review is mostly concerned with corticoids. Tamm reports mean values of about 40 $\mu\text{g./100 ml.}$ for dehydroisoandrosterone and about 20 $\mu\text{g./100 ml.}$ for androsterone and etioholanolone in the blood of men and women alike, less in children. Other recent human figures show 28-72 $\mu\text{g./100 ml.}$ total 17-ketosteroids and a mean for adult males of 171 $\mu\text{g./100 ml.}$ total 17-ketosteroids, of which 65% was dehydroiso-

androsterone and 11% androsterone (41, 207). Little is known about other species. It also seems clear that the above measurements refer mainly to adrenal steroids; testosterone is not a 17-ketosteroid. However, no information about normal circulating testosterone levels seems to be available.

Androgens are usually injected in oily solution in which they are more soluble than in water or saline, in crystalline aqueous or oily mushes (which delay absorption), or implanted as compressed tablets. The free compounds or esters may be used, esterification itself causing further delay in absorption. Testosterone propionate is the commonest injected androgen and needs to be given only every few days to maintain an effective circulating level of hormone. The actual amount of testosterone in the circulation at any one time after injection does not seem to have been measured, but it is clear that gradual absorption from a mush or tablet is much less wasteful of material than are repeated injections. Thus, injections of free or esterified testosterone to man and the domestic animals to repair castration changes are typically of the order of 0.5 to 1.0 mg./kg., repeated daily or every few days. On the other hand, 4-6 mg./kg. implanted every 3-4 months will serve the same purpose—a saving of some 90% of the injected hormone.

Dorfman and Shipley (63) also quote extensively from a review by Tornblom (237), in which earlier figures are expressed as $\mu\text{g./100 ml.}$ testosterone, but with no check on the actual nature of the androgen. Normal men and normal women differed little in blood content (mean $4.7 \pm 0.6 \mu\text{g./100 ml.}$; women $7.5 \pm 3.5 \mu\text{g./100 ml.}$) while, surprisingly, castrates of both sexes were much lower (approximately $1 \mu\text{g./100 ml.}$).

2. Estrogens

Estrogens produce general body metabolic effects different from those with androgens. A possible exception is the protein anabolic effect especially prominent in ruminants; this may be an indirect effect, however, involving the stimulation of the secretion of androgenic substances by the adrenal cortex (41a) or of the secretion of growth hormone from the anterior pituitary gland. Growth of bones is inhibited and ossification is intensified. This may cause sufficient narrowing of the marrow cavity to result in anemia, an effect particularly marked in birds, but seen also in mammals (223). In the dog, less prominently in other mammals, fatal anemia may follow which is not entirely due to bone marrow effects.

Another effect of estrogens is vasodilation and consequent edema, not confined to any one area, and apparently unaffected by innervation. The "sex skin" of some primates especially shows this phenomenon, and

following prolonged treatment for several weeks the edema may spread over most of the body surface, even in males (9, 79).

Estrogens affect the pituitary gland more profoundly than do androgens; a virtual chemical hypophysectomy is possible with continued high dosage, resulting in inhibition of growth and in gonadal regression. In rodents of both sexes, pituitary tumors may eventually develop and may cause blindness and death [for review of the earlier work see Burrows (29)]. The same reciprocal relationship exists between the ovary and the hypophysis as between the testes and hypophysis, but with much greater complexity in the normal cyclic female.

Mitotic activity and the action of steroids has been studied in particular by Bullough, who has recently summarized his views (27). Estrogens in particular stimulate the glucokinase system and it is believed that this is in all probability the main basis of their mitogenic actions. All estrogens tested have proved active, increasing mitosis in the general epidermis, as well as in the sex tract. Although other reports have indicated similar action with androgens (28), this general reaction is not now found with testosterone or progesterone.

Problems of behavior in the female are much more complex than in the male, and it is becoming more and more apparent that the term "estrogen" may in many species be a misnomer, in that psychic, if not also bodily estrus, is in fact more readily or even solely produced by combinations of estrogen and other steroids, notably progesterone, or even by androgen. The question is more fully discussed below.

Estrogens have recently been shown to act in an apparently normal manner, in tissue culture (112). Slices of rodent vagina respond by stratification and cornification in the course of a few days and show the characteristics of the *in vivo* response. While a great deal of biochemical work has been carried out on the interaction of steroids and enzyme systems *in vitro* (59), this is one of the first demonstrations of a full *in vitro* biological response, and is not shown except in the organized tissue slice.

A summary of recent work on blood estrogens is given by Christiansen (39). Peak values of about 6 $\mu\text{g./liter}$ are seen in the course of the menstrual cycle in women. More details of the normal cycle are given by Varangot *et al.* (238) who find $6.7 \pm 3.6 \mu\text{g./10 cm.}^3$ of combined estrone and estradiol in the first half of the cycle and $7.8 \pm 3.6 \mu\text{g./10 cm.}^3$ of combined estrone-estradiol and $0.75 \pm 0.80 \mu\text{g./10 cm.}^3$ of estriol. If 10 cm.^3 means liter, these figures are in line with the general literature, but if it means 10 ml., they are much greater than usual. In pregnancy, blood estrogens are elevated. Thus, Preedy (203) found in late pregnancy 2.7–10.0 $\mu\text{g./100 ml.}$ of estrone, 1.3–2.9 $\mu\text{g./100 ml.}$ of

estradiol and 4.3–17.5 $\mu\text{g./ml.}$ of estriol, a particularly striking difference since this author found only traces of all three in normal female urine (0–0.2 $\mu\text{g./100 ml.}$ estrone, 0.2–0.3 $\mu\text{g./100 ml.}$ estriol).

Estrogens are administered in much the same way as are androgens, being also relatively soluble in oils, but studies of estrogen levels in the blood following injection seem to be particularly lacking. Estrogen dosage varies enormously, as the compounds are used for such a variety of purposes, and rarely for simple repair of castration changes as in the male. However, doses are in general much lower than the doses of androgens, as it is typical of the natural and most potent synthetic estrogens that micrograms suffice where milligrams of androgens are needed. Thus, menopausal changes in women may be sufficiently corrected by daily doses of 0.025–1.0 mg. of stilbestrol or ethinylestradiol.

3. Progesterone

Except when given in high doses to the nonpregnant female, progesterone has less effect on general bodily functions than have androgens or estrogens. It acts somewhat like adrenal steroids in causing decreases in circulating eosinophils in adrenalectomized rats (43) but does not copy these steroids in many other ways. Various actions of this and other steroids on the extragenital system are summarized by Pineus (200).

More work has been done on blood levels of progesterone, including postinjection levels, than with most other steroids. The results are remarkably discrepant, however, and one is faced with no clear picture. In general, biological tests of the type first elaborated by Hooker (130) give much higher estimates than chemical or physicochemical methods. Thus, Forbes (87) found 5.2 $\mu\text{g./ml.}$ in the plasma in the luteal phase of the menstrual cycle, while Neher and Zarrow (189) found 0.3–2.0 $\mu\text{g./ml.}$ at estrus in the ewe, 6.0 $\mu\text{g./ml.}$ in the luteal phase, and a peak of 8–12 $\mu\text{g./ml.}$ in pregnancy. Zarrow and Neher (255) also found up to 10 $\mu\text{g./ml.}$ in the pregnant rabbit and 1.0 $\mu\text{g./ml.}$ normally. These authors used Hooker's technique. In contrast, Hoffman and Von Lam (129) found maxima of 0.004 $\mu\text{g./ml.}$ and 0.009 $\mu\text{g./ml.}$ in the luteal phase and in pregnancy in the human, respectively; Zander (252, 254) found 0.039–0.268 $\mu\text{g./ml.}$ in the last third of human pregnancy; Sommerville (232) found 0.098, 0.110, and 0.123 $\mu\text{g./ml.}$ in human pregnancy bloods and 3.76 $\mu\text{g./ml.}$ after the intravenous infusion of 150 mg. in the human. These authors used chemical techniques of estimates.

Raeside and Turner (206) injected progesterone subcutaneously to cattle, sheep, and goats—in cattle 1 g. gave about a 1 $\mu\text{g./ml.}$ peak after 2 hours; 100 mg., a 0.5 $\mu\text{g./ml.}$ in the same time, and again using chemical estimation. The daily injection of 1 g. in a heifer gave a level of

about 0.6 $\mu\text{g./ml.}$ in the peripheral blood, while daily injections of 100 mg. gave levels not much below this, indicating much wastage at the higher dose level.

II. ANDROGENS

A. *Normal Role at Puberty and the Breeding Season*

There is little evidence for significant secretory activity of the testis as an endocrine gland prior to the approach of puberty. While embryonic development may be disturbed by the administration of sex hormones, it still seems very dubious that such a mechanism is physiological, even in the production of freemartins (139). From excretion studies, it would appear that the human urinary content of 17-ketosteroids (which supposedly reflects the male hormone content of the blood), or of actual androgens, is so low as to be negligible in the normal subject until puberty commences, as shown in Figs. 5 and 6. Thereafter it rises rapidly and remains high until senescence. No corresponding data are available from any animal species, so it has to be conjectured that a similar course of events occurs.

Prior to puberty, the accessory organs can respond to sex hormones. It is thus reasonable to assume that the quiescent prepubertal sex tract is receiving no stimulus, since it could respond if it were. Puberty may be stimulated by normal testicular activity at a varying time in relation to body growth in different species. In most rodents studied, puberty occurs at about half the adult body weight, whereas in primates general body growth is much more advanced (7). Also, puberty and sexual maturity tend to be almost synonymous in rodents and other small animals, whereas in many of the larger domestic animals and man himself, sexual maturity follows only after an interval of months or years. We must therefore differentiate the two phenomena: puberty, the time when reproduction is possible, and sexual maturity, when full reproductive capacity is reached.

In animals which show little or no breeding season—and these are surprisingly few—the production of androgens continues at a presumably steady pace. In most species, but bred out to a varying extent in domestic animals, a breeding season is normal although less obvious in the male than in the female. Marshall's account of the breeding season (167) should be consulted for the almost infinite variety of cyclic activity offered by lower forms; we shall confine ourselves to vertebrates and largely to mammals. Various stimuli in vertebrates act on the hypophysis and cause gonadotropin release, changes in the diurnal period of illumination are common in higher vertebrates as a triggering mechanism. These changes are reflected by variation in gonadal hormone output and

in development of the reproductive tract, spermatogenesis, and sexual activity. Puberty occurs once, but sexual activity in many species recurs periodically.

B. Effects of Castration

The effects of castration have been known in part from antiquity, particularly as regards man and some of the domestic animals. The testes

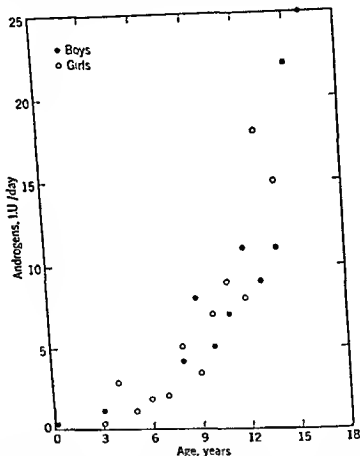


FIG. 5. Androgen excretion in childhood. Reprinted from Dorfman and Shipley (63), p. 396.

of all vertebrates would appear to secrete androgens; they contain the relevant cells (Leydig cells of the interstitial tissue) in fishes (44, 47), amphibia, reptiles (140), birds, and mammals. The androgens from fish testes stimulate comb growth in capons or chicks (118, 202) and it seems reasonable to suppose that they are of a similar chemical nature in all vertebrates. Similarly, synthetic androgens cause characteristic changes in fishes (128).

The main actions of male sex hormones are shown by changes following castration or by the failure to develop particular characteristics after

prepubertal castration Thus, from the lizard (173, 174) and bird (194) to man, castration is followed by failure to develop secondary sexual characteristics or their subsequent loss, by fat deposition in many instances, by the appearance in general of a neutral sexual condition, and the loss or absence of libido

Extensive work on castration and the effects of male hormone has been done in the domestic fowl Castration is followed in the cockerel by regression of the comb and wattles, by loss of the capacity to crow,

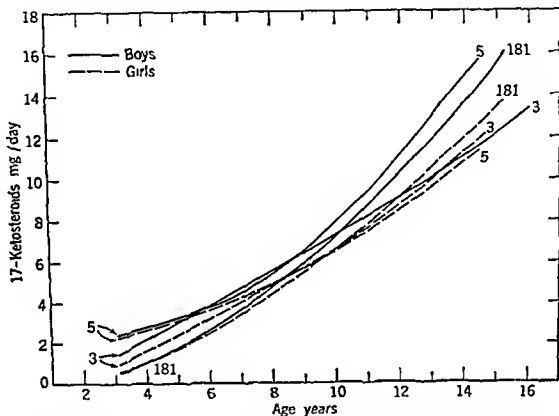


FIG 6 17-Ketosteroid excretion in childhood Reprinted from Dorfman and Shupley (63), p 397 (Numbers accompanying the smoothed curves indicate references in the original article)

involution of the vas deferens, loss of aggressive behavior, and, in some breeds, by assumption of a neutral plumage not previously seen In normal breeds, the plumage of the cock and the eapon is identical, but in the so called henny-feathered breeds (196) the cock responds to male hormone as does the hen to estrogens, and the two sexes thus have identical plumage On eaponization, the neutral plumage appears Castration of the chick prevents the development of male characteristics in the first instance The cooperation of androgens and estrogens rather than progesterone and estrogens seems to be of importance in the development of the sex tract of pullets (23)

In mammals, involution of various organs is seen if castration is performed postpubertally, or failure to develop is seen if it is performed

prepubertally. The development and maintenance of the penis, scrotum, prostate gland, seminal vesicles, Cowper's gland, vas deferens, epididymis, of the characteristic laryngeal changes and hair distribution in some species, such as man and the lion, and normal sexual behavior depend on the testes. The glandular secretions fail on castration. The seminal plasma in an early castrate stage fails to contain the characteristic fructose, citric acid, and other constituents (164), and in a later stage plasma itself is not produced. Male castrates typically accumulate body fat, a well-recognized fact in farm animals, and true also for such laboratory animals as the rat (147). However, clinical experience of men and animals shows that the castrate is not always fat and that the distribution of body fat is, in addition, only partly controlled by the gonads (110).

In both birds and mammals, castration affects the pituitary gland. The anterior pituitary gland increases in size (83), and changes profoundly in histology. The basophil cells increase in number and size and the acidophils decrease in number and appear to regress to chromophobes (116). Accompanying this, in some species, is a vacuolization of the basophils, resulting in the so-called signet-ring cell, or castration cell, with a large vacuole in the center and the nucleus looking like the signet in a ring of protoplasm (2, 225). In the rat, the normal pituitaries studied by Ellison and Wolfe (69) had 5.5% of basophils, those of castrates showed 17% of basophils, of which 1.4% were typical signet-ring cells. Another pituitary change is an increase in content of gonadotropin, mostly of follicle-stimulating hormone (FSH) activity (156). This increased production has been shown parabiotically (169) by biological assay of pituitary extracts, or by assays of serum or urine (37, 106). Urinary gonadotropin in castrated men may rise tenfold compared with normals.

C. Effects on the Male Reproductive Organs

1. External Genitalia.

In the rat, the penis hypertrophies with an excess of androgen, and androgen treatment prevents castration atrophy (148). Hypertrophy of the penis is expected when treating boys for undescended testicle with chorionic gonadotropin. An extensive survey of the effects of various androgens on the glans penis and the prepuce is given by Burrows (29). The musculature of the rat penis is particularly sensitive to androgens, for example; Wainman and Shipounoff (242) found that the weight of the perineal muscles after the injection of 500 µg. of testosterone propionate daily for 35 days increased from an average of 1.21 g. to 1.94 g.

The scrotum is similarly dependent on male hormone, and adequate treatment prevents the shrinkage and depigmentation that otherwise occurs on castration (107, 108). Twenty immature male rats given 19 daily injections of testosterone, commencing at 14 days of age, had scrota 1.5 to 3 times the weight of those of uninjected controls. Scrotal enlargement is also seen in boys treated for undescended testes by gonadotropin injections, due, as with hypertrophy of the penis, to excessive androgen secretion.

2. Internal Genitalia

The prostate gland is stimulated to both growth and secretory activity by androgens, and castration involution is prevented by androgen therapy (183). In some rodents, the immature animal's adrenal, with a persistent X-zone, may produce enough androgen to maintain prostatic activity to a marked extent (132, 133, 204). In the adult, however, with an involuted X-zone, castration is followed by full prostatic degeneration. This is mentioned because the need for, and effectiveness of, androgens in maintaining the prostate may seem uncertain with some experiments on rodents. Parabolic experiments by Martins and Rocha (169), in which two male rats were joined, showed that excess of androgen may cause prostatic hypertrophy. When one partner of two males is castrated, its own pituitary gonadotropin rises and the testes of the other partner are stimulated to secrete excess of androgen. This results in hypertrophy of the noncastrate's prostate gland.

The principal effect of androgens is on the secretory part of the prostate gland. Estrogens stimulate the stroma and thus a cooperative effect may occur (152). In addition, an anomalous result may be seen on giving weak androgens in older males, when an inhibitory effect on the pituitary output of gonadotropin may result in such a fall in endogenous secretion of androgen that an over-all lower androgen level is the result. When this occurs, the prostate and other glands may actually decrease in weight (150).

Prostatic secretion has been recognized as dependent on androgen for some time [(136) and many later papers by Huggins *et al.*]. Since the work of Mann and his colleagues, a good deal of attention has been focused on the biochemical side of this phenomenon, much of which has recently been reviewed by Mann (164, 165). Fructose is produced as a result of androgenic stimulation from the dorsal prostate and coagulating gland in the rat, from the ventral prostate in the rabbit, but by the seminal vesicles in man. In the dog, apparently no fructose is produced at all. Other components of the semen, such as citric acid and acid phosphatase, have been shown to be hormone-dependent (135, 163).

The coagulating gland was shown by Camus and Gley (34, 35) to release a specific enzyme causing coagulation of vesicular fluid and is distinct from the rest of the prostate in the guinea pig and some other rodents. This gland is often not separately mentioned when discussing the prostate. It is equally dependent on androgen for maintenance.

The seminal vesicles produce a fluid which clots on mixture with the products of the coagulating gland (102); this is responsible for the vaginal plug in many rodents. The degree of clotting varies with species, depending not only on whether a seminal vesicle is present, but also on the activity of the mixture. The enzyme just mentioned, vesiculase, acts on a protein from the seminal vesicles, strongly in the rat or mouse, weakly in man, hardly or not at all in the horse or bull. In most species the seminal vesicle is the main producer of fructose (164, 165). Androgens repair castration changes in these glands or stimulate precocious growth (187, 188); as with the other glands, secretion is dependent on androgens (166).

The preputial glands and Cowper's gland have been quite extensively studied, although the significance of their secretions remains in part obscure. They are androgen-dependent (179, 240) and subject to greater growth under simultaneous treatment with estrogen, as with many of the male secondary sexual organs. The epididymis and the vas deferens are likewise under the control of androgens, and castration changes may be prevented by experimental therapy (137, 181, 239).

The testis itself falls into a somewhat different category from the foregoing in that it is the site of production of the androgen concerned. Earlier workers tended to assume that its own secretion did not affect the testis except perhaps via the pituitary gland; now, however, a direct action on the seminiferous tubules is recognized. Moore and Price (186) showed that bull testis extract caused damage to the testes of immature rats, including the germinal epithelium, and suggest a pituitary influence. Later workers have shown that the exact effects produced depend on age, dosage level, the androgen used, duration of treatment, and species or strain of animals. The subject is well reviewed by Dorfman and Shipley (63). Most of the work has been on intact rats, and has shown that even 2 μ g. of testosterone propionate per day causes degeneration in the very young rat, but that much higher doses are ineffective at 70 days of age or more. Very high doses (30 mg. per week or more) produced testicular enlargement due to a direct effect (discussed below) in which the tubules are stimulated but Leydig cells degenerate. The most intense damage is with testosterone or methyltestosterone, at about 1 mg. per day, and is progressive with time up to 195 days (228). Similar

effects have been demonstrated in the guinea pig (18), and ground squirrel (218), but in the mouse a high and probably protective dose of 5 mg. per day did no damage (143), while no effect of high or low dosage (0.5 or 5.0 mg./day) followed Moore and Morgan's experiments with the opossum (185). This testicular damage is not always readily reversible, particularly if caused by early, high dosage.

The involvement of the pituitary gland is shown by decreased amounts of gonadotropin in glands from treated animals (187) and by the fact that testicular atrophy can be prevented by the simultaneous injection of gonadotropic hormone (18).

The direct effect of testosterone (or other androgens) on the testis was first demonstrated after hypophysectomy in the rat (243, 244). Degeneration of interstitial cells still occurred, but the seminiferous tubules were maintained. These experiments were amply confirmed by later workers, and the effect has been shown in the ground squirrel, pigeon, and primates. In the rat, spermatogenesis was best maintained by androstenediol rather than testosterone, which in Masson's (171) experiments failed to maintain it, while maintaining testis weight quite well.

These results illustrate a typical relationship between the pituitary gland and one of its dependent glands. Excessive production of the target gland's own hormone is followed by a decrease in the pituitary output of the tropic hormone concerned, and thus by a lowered output of the target gland and a new balance. In the case of the testis, a complication arises in that one of the tissues affected by testosterone is part of the testis itself—the seminiferous tubules.

D. Effects on the Female

Since the adrenal glands and perhaps even the ovaries secrete male hormone, the presence of a certain amount of circulating androgen is normal—in the human female, the total blood level of 17-ketosteroids and their excretion in the urine is about the same as in the male, but it is not clear how much represents original androgen.

In lower vertebrates, androgens may produce seemingly odd effects. Thus, the female South African clawed toad (*Xenopus laevis*) ovulates under androgenic stimulation (226), even the excised toad ovary doing so (227). The comb and wattles of the domestic hen grow when androgens are injected or inhaled, and various other characteristic androgen responses are elicited in other birds (249, 250). Androgens stimulate the oviduct, as do estrogens, and they also cause follicular stimulation (40, 227). In some ways, therefore, androgens act as gonadotropins in birds and amphibia.

In mammals, androgens affect the female reproductive tract in an important variety of ways. The uterus of spayed females is maintained in weight by the so-called metrotropic activity of androgens, affecting both the myometrium and the mucosa (149, 151, 190, 192). The estrogen-primed uterus may be used to show the progestational effect of androgens, which is never so pronounced as with progesterone itself (77, 141, 175). The progestational effect is fully developed in ethynyltestosterone (78) which is metrotropic, and androgenic and estrogenic, in addition, although it seems probable that each activity may be due to a different metabolite—the estrogenic activity certainly is (72). Ethynyltestosterone has the further advantage that, although it is less potent than progesterone, it is active by mouth. Another progesterone-like action of androgens is a dampening of uterine motility (214).

Premature opening and cornification of the vagina in rodents follow administration of some androgens, such as androstenediol (31, 70), subsequently shown by Rubinstein *et al.* (218) to be due to direct action of the compounds concerned. This is not confirmed by the tests of Emmens (72) in which the proestrogenic character of such androgens was established, so that although the pituitary or ovary may not be concerned, the androgens are not themselves the cause of the vaginal response; instead, a metabolite is presumably responsible. Androgens will normally prevent the vaginal cornification caused by estrogens (76, 215).

The female preputial glands and prostate (Skene's ducts) are affected by androgens, shadowing the corresponding effects on the male. Thus, glands of supranormal size may be produced and may, in the case of the prostate, secrete increased citric acid (205). Growth of the clitoris to a penis-like organ under androgenic stimulation is a well-known phenomenon, seen also in virilizing conditions in women. The female urethra may also exhibit hypospadias.

The ovary may be affected directly or via the pituitary gland. Most effects described fall into the latter class. Follicular maturation occurs in the rat, followed often by luteinization without ovulation (221)—a response abolished by hypophysectomy. This would appear to be consequent upon depression of LH output and stimulation of FSH output. In general, it would seem that short treatment with testosterone causes increased pituitary output of FSH in the rat or mouse, whereas longer treatment (several months) causes ovarian atrophy due to pituitary suppression. Again, this resembles the effects of progesterone. Noble (192) suggests a direct effect on the ovary in maintaining corpora lutea.

Androgens have been found to stimulate sexual desire in the human female—perhaps copying the effects of progesterone in this regard. Thus, patients treated with androgen for such conditions as breast cancer show

in most cases a heightened sex drive and easier attainment of orgasm (94, 222)

E Effects on the Embryo

Gallien (91, 91a, 91b, 91c) showed that, in *Rana temporaria*, genetic females may be completely transformed to males by administration of testosterone propionate to the tadpole or frog. The modified sex is stable and fertile, and is not reversed by treatment with estradiol subsequently. Not all amphibia respond as fully, and intersexuals may result, depending on species, dosage, time of treatment, etc.

In the chick, injection of developing eggs causes partial sex reversal, affecting testes and secondary sexual characters. Testosterone does not much affect males, but causes ovaries to become testis-like, and stimulates the Wolffian ducts of genetic females. Other androgens, such as androsterone, have a feminizing effect as well, and stimulate both the Wolffian and Mullerian ducts (182), androsterone also causes both testes and ovaries to become ovotestes.

In mammals, mainly the rat and the opossum, even less effect is seen, with no sex reversal or even hermaphroditism. Secondary masculine sexual structures are stimulated by androgens in both sexes, and again, derivatives of both the Wolffian and Mullerian ducts may be stimulated by androgen in the opossum (97). Gonadectomy of the early opossum embryo (182) does not affect development of the reproductive tract, but in the rabbit, Jost (139) found failure of Mullerian duct development if castration of the male fetus occurred before the 19th day. Various anomalous findings, and the absence of any very complete response the higher one ascends the vertebrate phylum, make it seem unlikely that the normal sex hormones are responsible for the embryonic development of the gonads or sex tracts. There is, in fact, no evidence that, whatever "hormone" the early embryonic gonad may secrete, it is testosterone or estradiol or a steroid at all.

F Assay

1 Biological Assay

Until recently there was an international standard preparation of androsterone, of which the specific activity contained in 0.1 mg constituted the international unit. On exhaustion of this stock, androsterone has ceased (or will cease) to be a standard, since the various androgens can be made synthetically. Androsterone is a common human urinary androgen, and was one of the first available in quantity.

Growth of the atrophic comb of the capon was first used as a method for measuring androgenic activity, and adapted as an assay method by

Gallagher and Koch (90) and by Greenwood *et al.* (100), later modified by McCullogh and Cuyler (176) and Emmens (71). In such a test, groups of capons are injected with oily solutions into the breast muscles, once a day for 3 to 5 days, and the resulting increases in length-plus-height (or over-all shadowgraph area) are measured one day later. When this increase is related to dose, or log dose, straight regression lines may be obtained from which relative potency may be obtained by standard methods (73, 85). This type of assay requires 1-2 mg. of androsterone (0.2-0.4 mg. of testosterone) per bird, and might prove too liberal for many types of inquiry.

Very much greater sensitivity is gained by direct application of the androgen to the capon's comb in an otherwise similar test. Responses then occur to 2-4 μ g. of androsterone or testosterone, which then elicits much the same weight-for-weight increase. The response is also somewhat more variable, but more birds can be used per group without demanding much material.

Responses of the chick comb were investigated by Ruzicka (219) and later by many others. The test may be by injection, or more usefully by direct application, as above (inunction). The comb is usually weighed after removal from the chick, and thus unstimulated controls must be used. The treatment period is also usually longer than with the capon comb. The test was exhaustively investigated by Dorfman (57); who concluded that errors within $\pm 38\%$ could be obtained with 32 chicks per substance in a 4-point assay (2 groups each on the standard and an unknown), within the range 20-160 μ g. of testosterone propionate.

Assays with mammals are preferred by many workers. Since they involve dissection, take more time, and more androgen, they have severe disadvantages. The methods are reviewed in detail by Dorfman (58). The type of assay evolved by Green and Burrill (95) is typical of most recent preference, being rapid and not too demanding of androgen. Immature rats, 20-22 days old, are given a single dose of hormone; 48 hours later the seminal vesicles are dissected and weighed fresh. The log dose-response line is straight over the range 5-50 μ g. of testosterone propionate; with 40 rats in an assay, the 5% limits of error (within which 95% of all results should fall) are approximately 65-160%. When castrates were used in an otherwise similar test (172), the 5% limits of error were 74-136%. Dorfman (60) and Emmens (75) may be consulted for recent bio-assay methods for steroids in general.

2. Chemical Assay

A great deal of attention has been paid, not so much to the chemical assay of androgens as such, but to the assay of 17-ketosteroids, which

include most androgens and their human urinary metabolites, whether androgenic or not. However, testosterone itself is not a 17-ketosteroid, although most of its metabolites probably are in the human. A correlation exists between the human urinary content of androgens and 17-ketosteroids (33).

The most common reaction for determining 17-ketosteroids after suitable extraction from the urine is the Zimmerman reaction, but many other methods have been investigated. For a general account Dorfman and Shipley (63) should be consulted.

III. ESTROGENS

A. *Normal Role at Puberty and the Breeding Season*

As with the testis, there is no good evidence that the ovary secretes steroid hormones prior to the onset of puberty. The human urinary content of estrogens is low prior to puberty, and that which occurs can reasonably be attributed to products of the adrenal cortex. Again, as in the male, the sex tract can respond to estrogens, and the absence of any response further argues their lack.

When significant ovarian secretion commences, it does so in a cyclic manner, continuing apart from pregnancy or anestrus until the breeding age is past. There is a sudden increase in ovarian size at puberty due to secretion of liquor folliculi, but the full development of estrous cycles is a gradual phenomenon, preceded by anovulatory and otherwise incomplete cycles. This characterizes the period of adolescent sterility, during which cyclic activity and the overt onset of puberty is accompanied by few or no fertile cycles. In the human, this phase may last for several years, and the menarche is not synonymous with fertility. Thereafter, full sexual maturity is gradually attained, although most animals are less fecund at first. In rodents and pigs, the first litter is smaller than subsequent ones and in sheep, the proportion of singles at the first lambing is very high (7).

In some species, the breeding season seems to be more sharply defined for the female than for the male. Thus, spermatogenesis in Merino rams goes on all the year round, but the ewes show a definite breeding season, although breeding does not cease completely in the free state at any time of year with good nutrition.

B. *Effects of Ovariectomy*

As with orchidectomy, ovariectomy is characterized by regression of, or failure to develop, secondary sexual characters. The younger the age of ovariectomy, the more profound the results are likely to be. All

vertebrates appear to secrete estrogens, and the effects of ovariectomy are similar in the different classes. Attempts have been made to extract ovarian hormones from fish (56, 82) and small amounts of estrogen have been found. Fish urine contains substances suggestive of estrogenic steroids, but their identity has not yet been established (24). Thus, even so far down the vertebrate scale, estrogens exist, but may not be steroids. In the female as in the male, however, steroid estrogens of mammalian or synthetic origin act in fishes as do the natural hormones (55).

In birds, ovariectomy, besides affecting breeding behavior and the reproductive tract, may affect the plumage, causing the hen to revert in many breeds of domestic fowl to the neutral plumage of the capon or poularde. Another rather anomalous result in this class may be the activation of the right gonadal element, which may grow to become an ovotestis or even, in extremely rare cases, a functional testis. The same result may follow naturally after extensive ovarian disease.

In the mammal, spaying of the immature female results in failure of development of the Fallopian tubes, uterus, vagina and accessory glands, of the mammary glands and teats, and of the typical female bone formation and fat distribution in many species. Regression towards the neutral type occurs in postpubertal gonadectomy. A natural "spaying" occurs over the somewhat extended period of the menopause in the human subject, and is accompanied by various regressive changes, as indicated above, sometimes also by psychotic disorders.

Gonadectomy affects the pituitary gland, which increases in size and gonadotropin output, but the size change would appear, in some species at least, to be less than is seen with the male (116, 117). There is in particular an increase in FSH activity, again as in the male (256), and the urinary gonadotropin of postmenopausal women is mainly FSH in nature.

Mention has been made of the adrenal cortex as a source of estrogen, and it is interesting that in old female mouse castrates, sufficient estrogen may come from this source to cause activation of the reproductive tract (251).

C. Effects on the Female Reproductive Organs

The effect of estrogens on the ovariectomized female rodent in causing cornification of the vagina has overshadowed their other activities to an almost alarming extent. Thus, many a compound pronounced to be estrogenic has never been tested except by this one criterion. The test, fortunately, appears to be very specific.

In discussing the effects of estrogens, therefore, it has to be recalled that vaginal cornification is not synonymous with the production of true estrus or readiness to mate, which often depends on the interaction of hormones rather than the effects of any single compound. In the mouse, for instance, only very few animals respond to cornifying or even very much higher doses of estrogen by a simultaneous readiness to accept the male.

Estrogen administered to castrates prevents atrophy of the vagina and, if in high enough dosage, causes rodent vaginal cornification or changes similar to it in other species, thus providing the test referred to above, elaborated by Allen and Doisy (4, 5) on the original observations of Stockard and Papanicolaou (233). A full discussion of the earlier work and of the detailed changes seen under estrogenic treatment is found in Burrows (29). Glycogen deposition follows estrogen treatment (14, 64) and may be responsible via lactic acid production for the lowered pH which also occurs (199). The initial response of the vagina appears to be mitotic, followed by the development of stratification and mucification and finally cornification of the inner vaginal layers (those layers farthest from the basement membrane) in some species (13). These phenomena have been duplicated *in vitro*, using slices of mouse vagina, and are apparently independent of blood supply or of other hormones (112).

The early response of the uterus is a hyperemia, followed by water uptake (8, 45), and then a hypertrophy of both myometrium and endometrium (247). The water uptake reaches a stage of frank edema, particularly in rodents, but the same sequence of events is seen in all species—in normals, castrates, and hypophysectomized animals alike (230). A phase of glycogenesis occurs with glycogen deposition (17)—an effect which has not yet been produced *in vitro*—and it is interesting that glycogen deposition in the primate uterus appears to depend on progesterone instead.

Activity of the uterus is much affected by estrogen, which causes both spontaneous activity and activity resulting from sensitivity to posterior pituitary oxytocin to increase (210). The effects vary to some extent in different species, being much more striking in the rabbit and mouse than in many others. In these species, both *in vivo* and *in vitro*, spontaneous contractions and sensitivity to oxytocin increase under estrogenic treatment. Estrogens also prepare the uterus for the action of progesterone, which alone produces little effect.

Prolonged treatment with estrogens causes various uterine disturbances, starting with metaplasia and proceeding to pyometra, adenoma, and cancer (29). Infertility is naturally a concomitant result, and one

which attained economic importance recently in Australia, where the widespread use of early subterranean clover was associated with dystocia and infertility in sheep (11). Later, the clover was found to contain large amounts of genistein, a proestrogen.

Remarkably little work has been done on the effects of estrogens on the Fallopian tubes, although, as will be clear when the events accompanying the estrous cycle are discussed below, a cyclic influence on tubal structure and activity occurs.

The ovary itself is affected, as is the testis, via the pituitary gland. Estrogens are much more potent than androgens in suppressing gonadotropin output, and also the output of other anterior pituitary hormones. They may be used to produce a state closely resembling hypophysectomy if given in sufficient dosage for a period of time. However, the first effect of physiological doses is an apparent release of LH (155), but on continued administration general suppression of both LH and FSH output occurs. Again as with androgens, a direct gonadotropic action of estrogen itself may be demonstrated in suitable circumstances. Thus, Williams (246) found that diethylstilbestrol prevents the atrophy of the ovary, which normally follows hypophysectomy in the immature female rat, and increased the response to gonadotropin (PMS). The same treatment did not affect the ovary weight of normal controls. Payne and Hellbaum (197) have obtained similar results with a series of estrogens. A four-fold increase in ovarian weight above controls was obtained with diethylstilbestrol in hypophysectomized rats by Pencharz (197a). Reviewing the influence which estrogen secretion by smaller follicles has upon the definitive follicle led Allen (4a) to paraphrase, "The 'favored follicle' truly 'stands upon the shoulders of its contemporaries.'"

While it is possible that pituitary or other gonadotropins maintain the ovarian follicles by the local production of estrogen, this seems unlikely, since the greater part of the estrogen produced by the ovary comes from the follicles themselves, and the situation is not quite the same as with the testis, where the interstitial tissue may support tubular growth. On the other hand, follicular growth in the presence of excess systemic estrogen is definitely inhibited by suppression of the release of pituitary gonadotropin.

The effects of estrogens on the mammary gland are dealt with at greater length in Chapter 16. It is sufficient to note here that in some species estrogens alone cause mammary growth and even lactation, while in others progesterone and possibly other adrenal steroids are needed for full mammary development. Folley's recent book on the subject (86) should be consulted for details.

D. Effects on the Male

The estrogen output from the adrenal cortex means that, as in the female, a balance of androgenic and estrogenic activity normally exists in the male, so that he is accustomed to a certain level of estrogenic stimulation. In some cases, such as the stallion, whose testes produce large amounts of estrogen, the normal male is adapted to a very high level indeed.

The general effects of estrogens on males of both lower and higher vertebrates are feminization, with suppression of male secondary sexual characters and behavior. In the male, as in the female, a state of "chemical hypophysectomy" may be achieved with prolonged dosage. In birds, the plumage assumes the female character, and other secondary organs, such as the comb and wattles of the cock, regress. "Chemical caponization," a milder form of the condition mentioned above, may be achieved in cockerels or male birds of other species by regulated dosage with estrogens. Usually, a pellet is implanted into the neck.

The male mammal suffers decalcification of the pubic symphysis and resultant hernia under prolonged estrogen treatment (30, 93), testicular atrophy, and then atrophy of the secondary reproductive tract and sterility (241). These effects are reviewed in detail by Emmens and Parkes (79). The dosage of estrogen required in any one species to cause a particular effect varies widely; thus, mice are much more resistant than are rats and rabbits. Varying strains of mice have also been studied with reference to hyperplasia of the Leydig cells in the testis. This occurs with Strong albino mice quite readily on prolonged dosage with estrogens (29), but not in other strains, and is another aspect of effects on the pituitary gonadotropin output, which varies in different strains and thus causes resultant varying degrees of androgen production and protection from the results of estrogen dosage.

The ejaculate is reduced in volume by treatment with estrogens. Huggins and Clark (134) reported that, in dogs, 0.6 mg. or more per day of diethylstilbestrol caused a rapid fall in sperm count and in ejaculate volume to nil or almost nil within a month. Similar effects have been reported for boars and men, although with 4-5 mg. per day absorbed from tablets, two Suffolk rams treated by Chang (38) remained fertile and rose in sperm production at first. Later, sperm production fell below controls and would presumably have fallen further if the experiment had been continued.

As indicated above, males and females chronically dosed with estrogen, particularly if from early life, may be rendered not only sterile and eunuchoid, but also present a state resembling hypophysectomy. That this is due to a general pituitary suppression may be demonstrated by

injecting growth hormone, when growth is resumed. The "plateaued" rat, with a body weight of about 120 g. and exhibiting no further growth, is in fact used as a test for growth hormone. Even regression of the thyroid gland has been reported (12), so that the degree of "chemical hypophysectomy" may be quite advanced. However, there is no exact parallel, for hypophysectomized rats themselves "plateau" at about 70 g., and there is good evidence that estrogen directly inhibits bone growth as well as doing so via the pituitary gland.

Direct changes in the accessory male reproductive organs also follow estrogen treatment. Estrogen causes stratification and metaplasia of the urethral epithelium (184), muscular growth in the seminal vesicles (88), and enlargement of the prostate gland (154). Much work has been done in various species, particularly since estrogen treatment has proved beneficial in prostatic enlargement or carcinoma. It would seem that Müllerian elements enter into the structure of tissues showing an epithelial response, and that the inhibitory effect of estrogen on structures arising from the Wolffian ducts is not in disaccord with this view (257, 258).

The mammary gland of the male shows a remarkable capacity to respond to estrogens, and has been used extensively for studies of the effects of these hormones on mammary development (79, 86).

The normal male is much more resistant to the effects of estrogens than is the castrate. This is because his androgens protect him from some of the effects, and are locally antagonistic to the action of estrogens. Castrates may be protected by injection of androgens simultaneously with estrogens. Such inhibition requires very much more androgen than estrogen. Thus, de Jongh (138) found that about 300 μ g. of androsterone would inhibit the effects of 1 μ g. of estrone on the prostate gland of rats. Emmens and Bradshaw (76) found that 500 μ g. of testosterone and some other androgens were needed to inhibit the response of spayed mice to 0.12 μ g. of estrone. The reverse phenomenon, the inhibition of androgen by estrogen, occurs with a much more even ratio. Thus, the comb growth produced in Brown Leghorn capons by 600 μ g. of injected androsterone could be 50% inhibited by about 2 mg. of estradiol and totally inhibited by about 10 mg. This is in line with the much greater amounts of androgenic steroids needed physiologically, as compared with estrogenic steroids.

E. Effects on the Embryo

The embryos of lower vertebrates are more affected by estrogen treatment than are those of mammals. In amphibia, estrogens induce ovary development in both sexes (208); in the fowl, the same result is seen

(even though androgens do not, in the fowl, cause full testis development) Thus, Gallien (92) obtained 100% of female newts (*Pleurodeles waltli*) by keeping the tadpoles in estradiol benzoate solution (600 $\mu\text{g/l}$), and the adults laid eggs. The left testis more readily becomes an ovary in the fowl (90), but even the right gonads may be feminized (49, 50). However, fertility does not seem to have been reported in such birds. The derivatives of the Mullerian ducts (oviduct, etc.) are stimulated and those of the Wolffian ducts are repressed, but, as the birds grow up, there is a tendency for reversion to a more normal type in the genetic males.

In the mammal, attempts to produce feminization depend on failure to cause abortion or fetal resorption. Greene *et al* (96, 98, 99) injected pregnant rats with estrogens on the 13th, 14th, and 15th day of gestation and caused varying degrees of male hypospadias, visible nipples at birth, and abdominal testis and vaginal development, with inhibition of development of seminal vesicles and prostate, right vas deferens, scrotum, and penis. However, in these and many other similar studies, the male gonad has never been modified, and, as with treatment of the female with androgens, we must conclude that the sex hormones of the adult are not the determining factors in embryonal gonadal development in the mammal.

F Assay

1 Biological Assay

The international unit of estrogenic activity is that exhibited by 0.1 μg of international standard estrone (which approximates to a mouse unit of the earlier workers). A second international standard is estradiol benzoate, of which 0.1 μg is also the unit, this is intended for the assay of esterified substances. Both seem due for abolition on the same grounds as for androsterone.

Cornification of the rat or mouse vagina has, as remarked above, come to be very nearly synonymous with estrogenic activity. Recent reviews by Emmens (74, 75) and Dorfman (60) may be consulted for details. A typical Allen-Doisy test (5) employs groups of ovariectomized mice which are injected subcutaneously with an aqueous or oily solution of the estrogen under test, normally in comparison with a standard. Several injections are given over a period of 2 days, and vaginal smears are taken on the evening of the 3rd day and several times on the 4th day. These are scored as positive if they contain no leucocytes and many cornified epithelial cells, otherwise, as negative. The results are expressed as percentages of mice reacting, or as a function of these, and a test employ-

As do the other steroids so far considered, progesterone depresses the gonadotropic output of the pituitary gland. The amounts required and the activity seen resemble those of androgens rather than estrogens. Herlant (123) administered progesterone to adult female rats, removed the pituitaries at varying periods, and implanted them into immature females. Implants from rats receiving 1 mg. daily for only 4 days caused ovarian and uterine enlargement greater than in controls, but implants from rats receiving only 0.5 mg. per day or less for 10-14 days caused no such growth. This is interpreted as an increased LH production at first, followed by suppression. Other investigators have produced similar results in long-term tests.

The effect of progesterone on the ovary may thus be both direct and indirect. Despite Herlant's finding in short-term progesterone tests, it is generally agreed that progesterone suppresses ovulation, presumably by suppressing LH output. The earlier work commenced with Loeb (158), who found that extirpation of guinea pig corpora lutea led to early ovulation, and later investigators have found that daily doses of progesterone inhibit ovulation and estrus in rats, guinea pigs, and rabbits (51, 161, 162, 198).

Cooperation between progesterone and estrogens in producing psychic estrus is probably normal, but the exact relationship between the two hormones varies from species to species. At one extreme, the rat, mouse, or guinea pig requires an estrogenic stimulus followed by progesterone to produce sexual receptivity; at the other, a relatively long course of progesterone in the ewe is needed before a full response can be obtained to estrogen itself (212). This explains the phenomenon of "sileot heat" in that species, since the first estrous cycle of the season does not elicit receptivity in the absence of progesterone pretreatment, but subsequent cycles do so because of the preceding corpora lutea.

B. Normal Role in the Pregnant Female

In pregnancy, there is essentially a continuation of the progestational phase of the estrous cycle. Progesterone is essential for the maintenance of pregnancy in all its stages, and its withdrawal is followed by absorption, abortion, or premature birth. The source of progesterone during pregnancy varies in different species, being mainly ovarian in the early stages and mainly placental in the later stages of pregnancy in most domestic animals. At one extreme, animals like the mouse or goat provide so little placental progesterone that ovariectomy is followed by abortion at all stages of pregnancy, whereas in the mare or female primate, at the other extreme, only early removal of the ovary causes

abortion and a fully functional corpus luteum is not present in late pregnancy (7). Progesterone injections or corpus luteum extract will prolong a normal pregnancy if given when birth is to be expected (191). This depends on the maintenance of a functional placenta by progesterone and also on the prevention of responses to estrogen and oxytocin, which normally occur at birth. Thus, in preparing the uterus for implantation, in maintaining the integrity of the placenta and its relationship with the uterus, and in maintaining uterine growth (together with estrogen), progesterone plays its role throughout pregnancy.

While the events in the uterus are proceeding, mammary growth also occurs. It has been mentioned that different species show varying degrees of completeness of mammary development in response to experimental treatment with estrogens. In general, it is concluded by Folley (86) that normal mid-pregnant morphology (by which time the greater part of mammary growth is complete) is shown only on treatment with both estrogen and progesterone, but that three main categories of animal exist. The first category includes the domestic ruminants and the guinea pig, in which functional mammary glands can be developed by estrogen alone. The second includes the rat, rabbit, and cat, in which although both duct and alveolar growth can be developed by sufficiently prolonged estrogen treatment, full alveolar growth typically depends on progesterone. The last category, represented so far only by the bitch, shows little or no mammary development from estrogen alone, not even duct development. Even in the first category, it seems certain that normal mammary development depends in part on the action of progesterone, and experimentally in cows, combinations of estrogen and progesterone give more normal-looking glands than estrogen alone (46) and glands which secrete more milk (209).

C. Assay

1. *Biological Assay*

The assay of progesterone is still in an unsatisfactory stage. This is partly because the chemical assay of pregnanediol, an inert excretion product of progesterone in human urine, gives a satisfactory index of progesterone production in man. There has therefore been less pressure to develop satisfactory methods for progesterone itself. The response of the primed rabbit uterus is employed in the McPhail test (178), and provides rough estimates. However, 1-2 mg. of progesterone is needed per rabbit.

Hooker (130) has developed a test depending on hypertrophy of the stromal nuclei on local administration in the mouse uterus, which requires

ing a total of 80-100 mice gives limits of error in the neighbourhood of 77-130% for 5% fiducial limits of error.

If the estrogen is given intravaginally, a very sensitive test results (160) in which, contrary to the subcutaneous test, the various natural estrogens and the more potent synthetics are very much alike in potency (72). Thus, approximately 3×10^{-4} μ g. of estradiol, estrone, estriol or diethylstilbestrol all elicit 50% of positive responses. In a typical test, two intravaginal applications in water, saline, or up to 100% propylene glycol or glycerol may be given each on one of two consecutive days; smears are taken about 12 hours earlier than in the subcutaneous test. Not only are various estrogens alike in potency in the intravaginal test; they also appear unaffected by esterification, and thus an over-all rough guide to the amount of estrogen in a preparation from natural sources may be obtained readily with an intravaginal test.

More recently, the incidence of vaginal mitosis has been used as the basis of a further, very sensitive intravaginal test (168). In this test about 8×10^{-5} μ g. of estrone or estradiol is effective, a further advance on the intravaginal test as previously carried out. In tissue culture 4×10^{-6} μ g. is effective on a vaginal slice, under conditions where none of the estrogen can escape, and so it seems clear that the most sensitive intravaginal tests are approaching maximal possible sensitivity with the indices used. The very high sensitivity claimed by Sulman (234) in intravaginal tests, although only in special mice, is based on a response scored as positive if the number of leucocytes in a smear is less than the number of epithelial cells and does not compare directly with the above.

Another frequently used method for the bio-assay of estrogens is the increase of uterine weight in rodents. Astwood's (8) 6-hour test depends on water uptake by the uterus, longer tests such as those of Bulbring and Burn (26) on tissue weight increases. Recent work favors relatively short 1- or 2-day tests. The chick oviduct is increasingly used where convenient as an assay subject, but requires more hormone per assay (60). Neither uterine nor oviducal changes are as specific as vaginal cornification, nor as undisturbed by impurities in extracts.

Bio-assay methods appropriate to long-acting preparations, and based on duration of action, are discussed by Diczfalusy *et al.* (54).

2. Chemical Assay

Much recent work has been done on the chemical separation and assay of human urinary estrogens, with the result that the three compounds originally thought to be concerned (estrone, estriol, estradiol) can be separated and individually measured (52, 53). However, it is now known that other compounds are excreted by the human subject, and

more work remains to be done. None of this has great relevance to other species, in which the urinary estrogens or their derivatives may be quite different. In the urine of the mare, for example, a series typified by equilin and equilin (Fig. 3) is excreted, with different chemical properties.

Estimation, once chemical separation has been made, is still usually performed by one modification or another of the Kober reaction (144) or by fluorimetry. The Kober estimation is performed colorimetrically, and is sensitive to as little as 0.1 μ g. of a typical estrogen, but the fluorimetric method appears to be about 10 times as sensitive (22, 84). A number of other chemical and physical methods has also been tried.

IV. PROGESTERONE

A. Normal Role in the Nonpregnant Female

The main source of progesterone in the nonpregnant female is the corpus luteum, which develops cyclically in the ovary and releases progesterone for a period that varies in different species. Progesterone is normally released in quantity after a period of estrogenic stimulation, and many of its effects are not developed unless the animal has been "primed" with estrogen in this manner. This hormone has been little investigated as regards its action on the male or on the embryo, but in general it acts rather like an androgen.

In the adult female, progesterone causes vaginal mucification, the characteristic progestational phase of the estrous cycle shows a stratified, mucus-saturated epithelium. Ovulation and estrus are suppressed (195). Responses to estrogen are themselves suppressed, even 2 mg. of estrone per day may fail to cause cornification in the rat in the presence of corpora lutea of pregnancy. This has been shown to be due to the progesterone produced by the corpus luteum; thus, Selye *et al.* (224) found that 400 μ g. of progesterone abolishes the rat's response to 30 μ g. of estrone, the animals showing only mucification of the vagina.

The outstanding effects of progesterone are seen on the uterus. After a certain amount of response to estrogen, the uterus is in a state to undergo proliferation under the influence of progesterone, which particularly affects the endometrium. It greatly enlarges, and the tubular glands increase in depth and tortuosity. Uterine motility is decreased and the response of the uterus to estrogen and to oxytocin from the posterior pituitary gland is decreased or abolished (153, 213). A typical reaction seen only in rodents and primates, the deciduoma reaction, develops as a response to foreign body stimulation of the endometrium. This is normally elicited by the nidating ovum, or experimentally by slight injuries to the endometrium (157). A nodule of decidual tissue forms, which encloses the ovum in the course of implantation.

as little as 0.0002 $\mu\text{g.}$ of progesterone per mouse. Adult female mice are ovariectomized and used after 16 days, when a small amount of test or control solution is placed in a ligated uterine segment and the effect assessed 2 days later. The test has never been extensively investigated and false negatives have been reported in the presence of estradiol (220).

2. Chemical Assay

Some promising chemical methods have been described for the estimation of progesterone in blood. Apart from a method using a polarographic estimation (32), other methods rely on the ultraviolet absorption band near 240 $\text{m}\mu$ characteristic of $\alpha\beta$ -unsaturated ketones (68, 115, 211, 253, 254); a recently proposed technique estimates progesterone as the isonicotinic acid hydrazone which has an absorption maximum at 380 $\text{m}\mu$ (232). The reliability of these relatively nonspecific methods is dependent on the purification of extracts free of related compounds and interfering substances before the estimations are carried out.

V. THE ESTROUS CYCLE

✓ A. General Considerations

The nonbreeding or resting season in the mammalian female is referred to as the anestrus period or anestrus (often anestrus). It is of variable length, often occupying most of the year. It is followed by the breeding season, when one or more estrous cycles occur, which are divided up into the following phases (assuming no pregnancy to occur):

1. *Proestrus* is the start of the cycle, with a predominance of stimulation by pituitary FSH, the growth of ovarian follicles, and the increasing secretion of estrogens, possibly together with progesterone. The vagina and uterus grow and become hyperemic.

2. *Estrus* is the period of sexual receptivity, during which ovulation typically occurs and the corpus luteum starts to form. It is marked by a greater proportion of LH from the pituitary gland and a lowering of estrogen output when the follicle has ruptured.

3. *Metestrus* is the postovulatory phase, during which the corpus luteum may function for a varying period of time in different species, presumably depending on the time for which the pituitary gland puts out luteotropin, probably identical with prolactin. There is a decline in estrogen output (in the human, another brief peak) and an increase in progesterone secretion in most domestic species.

4. *Diestrus* is the period of relative quiescence before another cycle occurs. It is usually short in duration in polycyclic animals (i.e., those having recurring cycles). However, some authors define diestrus as an abbreviated pseudopregnancy, including the luteal phase in it.

This terminology was proposed by Heape (119) and is still most commonly employed today. However, Heape called it the diestrous cycle, whereas estrous cycle is now the commoner term. A very full discussion will be found in Asdell (7) and in Eckstein and Zuckerman (66).

In consequence of the typical activity exhibited, the proestrous period is often referred to as the follicular phase and the metestrous (and/or diestrous) period as the luteal phase. As will be seen from earlier parts of this chapter, the basis of the estrous cycle is a pituitary gland rhythm. There is, however, a series of interactions between the ovaries and pituitary gland, which reinforce the pituitary rhythm and perhaps modify it, even in the nonpregnant animal. Estrogen from the growing follicles may cause the accelerated release of LH and suppression of FSH; progesterone from the corpus luteum may, as discussed above, at first reinforce this action but later cause suppression of LH release, thus helping to initiate a further cycle. The length of life of the corpus luteum is also dependent on luteotropin, about the release or suppression of which comparatively little is known.

It is most generally thought that FSH alone (if indeed FSH can exist alone) does not cause the release of estrogens from the ovary. (In the hypophysectomized cock, FSH causes testicular enlargement but no comb growth and thus no androgen release.) It must be supposed that during follicular maturation an increasing amount of LH is also released from the pituitary gland, even if accompanied by increasing amounts of FSH as well. Then, after ovulation, which may be supposed to occur when the proportion or absolute level of LH exceeds a certain threshold, the circulating LH drops. It is possible that the level of FSH remains constant. The third pituitary gonadotropin, luteotropin, then may or may not act for a varying period, and cause prolongation of the life of the corpus luteum. It is also now most generally thought that LH, although it triggers the formation of the corpus luteum, is not responsible for progesterone production, at any rate in the absence of luteotropin.

If copulation occurs, it may modify the course of events, either by causing pseudopregnancy in some species or by causing pregnancy. Pseudopregnancy, seen especially well in the rabbit or bitch, is, as its name suggests, a prolonged period resembling pregnancy and accompanied by a number of changes in the reproductive tract and even in the behavior of the female. It is characterized by a prolongation of life of the corpus luteum and suppression for a period of further estrous cycles. It may be followed by estrous cycles, or by anestrus. Pregnancy is accompanied by the same events in the earlier stages as occur in pseudopregnancy, but the presence of a growing embryo and its appendages, particularly the placenta, soon causes other and different

events. Both, however, have in common the prolonged luteal phase with suppression of estrous cycles and usually of ovulation. The phenomenon of pseudopregnancy is of importance in appreciating the relationship between different types of estrous cycles.

B. Cyclic Changes in the Female Reproductive Organs

Accounts of the cytology and development of the ovary in mammals will be found in Wilson (248) and Brambell (20). The mammalian oocyte contains little yolk and typically reaches only 0.1 mm. in diameter, but lies in a ripe follicle of considerably larger dimensions. As the follicle approaches maturity, it rises to the surface of the ovarian cortex and protrudes from its surface, thus giving the appearance of even more rapid growth than is the case. The antrum develops after the ovum has reached its maximal size, when the follicle is still only about 200–400 μ in diameter in different species (21). On antrum formation, the follicle falls under the influence of gonadotropins, but development to that stage proceeds without them. Distention of the follicle follows from the secretion of liquor folliculi, which in the mature follicle is very viscous (122), flows from the follicle for some time on ovulation, and then forms a clot (245). The size of the follicle at maturity varies from about 0.3 to 3.0 cm. in diameter, in different species, the larger follicles occurring in larger mammals (20). When the follicles mature, the secretion of estrogen is about maximal, and this marks the termination of proestrus in most mammals.

Growth of the follicle prior to ovulation is very rapid, and the phenomenon of ovulation itself appears to be simple rupture as a consequence of increasing pressure, but opinions vary. There is a typical number of ripe follicles at each estrus—one in man or the mare, many in rodents or other animals bearing large litters. There may, however, be tremendous wastage; the record is apparently held by *Elephantulus*, in which about 60 ova are liberated from each ovary but only two can survive (131). After ovulation, and the passing of the estrous phase, metestrus is characterized by the formation of the corpus luteum from the wall of the follicle. Harrison (113) has fairly recently reviewed the literature on the corpus luteum very thoroughly. The fully formed organ consists of the large, yellow, luteal cells, in a framework of blood vessels. Apparently, similar bodies are seen in lower vertebrates, such as elasmobranch fishes, amphibia, and reptiles, but in most groups they regress from the start. Only in mammals is an endocrine function certain, and in some of these regression is prompt. As mentioned above, the corpus luteum persists in different species for a varying time, depending on the particular mammal and on other factors, such as pseudopregnancy.

pregnancy, and lactation. Thus, in the rat (159) in a normal estrous cycle, the corpora lutea show signs of regression at the time of onset of the next estrous period. Those formed after a sterile mating look the same at first, but the onset both of regression and of the next estrus is delayed for about 13 days. Those formed in pregnancy last correspondingly longer. However, even the short-lived corpora lutea of the unmated rat are visible as degenerating structures for several subsequent cycles, and it is the time for which the organ is actively secreting which determines the length of the metestrous phase. Details of the course of events in various species may be gathered from Asdell (7) and Brambell (20).

The changes in the accessory reproductive organs at various stages of the estrous cycle are reviewed by Asdell (7) and by Eckstein and Zuckerman (67). Vulval and vaginal changes vary according to species, and no domestic animal shows quite the sharp pattern obtainable by vaginal smear from the laboratory rodents. In the rat or mouse, the diestrous smear is full of leucocytes, with perhaps a few nucleated or even cornified epithelial cells, and a little mucus. At proestrus, the leucocytes disappear and the smear consists of masses of at first nucleated and then non-nucleated, cornified, epithelial cells. In late estrus, leucocytes commence to infiltrate the smear again, and during metestrus and early diestrus, they invade in large quantities [see Hartman (114) for a recent account].

A well-defined cycle occurs in the vagina of the bitch, with stratification and perhaps cornification at estrus (6, 80); leucocytic infiltration follows. In the cow, the vaginal mucous membrane undergoes some changes, particularly near to the cervix, but smears show little or nothing; mucus secretion is, however, characteristic of heat. In the ewe, also, the vaginal smear shows no such marked changes as in the rodent, although according to some authorities sufficient is seen to diagnose the stage of the cycle (42).

Uterine changes in rodents are very pronounced. During proestrus the uterine horns become distended with secretion and the epithelium becomes cuboidal. Astwood's test (8) based on the water uptake has already been mentioned. At early estrus, the water content falls abruptly and there is a weight drop. Then there is epithelial degeneration continuing into late metestrus. Proestrus in the bitch is characterized by intense hyperemia with bleeding from subepithelial capillaries (80), but no such great water uptake and loss as in the rodent. There is, however, edema, continued into estrus. A rather later (estrous) bleeding of a similar nature is seen in the cow. No bleeding occurs in the ewe, and much mucus production is seen at all stages (42), but the early uterine changes otherwise resemble those in other species. The biggest species

difference occurs in the metestrous plus diestrous period, when the length of functional life of the corpus luteum determines whether there is almost no luteal phase or a phase of uterine, particularly endometrial growth. This is minimal in rodents like the rat, lasts for 2-3 days in the mare, with the epithelium highest 2 days after the end of heat (111); for about a week in the ewe, which shows growth and coiling of the endometrial glands (177), and for about 2 weeks in the cow with correspondingly greater development (48).

In the cat and dog, the pseudopregnant luteal phase lasts for about a month, so that in the normal bitch (but only the mated cat) even more extreme luteal development may occur. In general, changes in the Fallopian tubes reflect those in the uterus to a certain degree, being claimed as more definite in the ewe than those in other parts of the tract (36).

C. *Factors Affecting the Type of Estrous Cycle*

There are three main types of estrous cycle seen in the laboratory and domestic animals:

1. Short 4-6 day cycles occur in rats and mice if no copulation is permitted. These seem to be almost purely follicular in nature, with little secretory activity of the corpus luteum. However, if sterile mating occurs, pseudopregnancy results and lasts for about 2 weeks, during which progestational activity of the corpus luteum is apparent. The stimulus of sterile mating can be copied by mechanical stimulation (159) or electrical stimulation (229) and a variety of other procedures.

2. Longer cycles of 16 or more days characterize the majority of domestic mammals and resemble the pseudopregnant rat cycle in many ways. Such species do not require an additional stimulus to produce fully functional corpora lutea, and sterile copulation does not modify the length of the estrous cycle.

3. Some species, such as the ferret and rabbit, and almost certainly the cat, do not ovulate without a mating stimulus, and the estrous cycle halts at estrus for a considerable period in the absence of copulation (about 2-4 weeks is seemingly typical). Coitus is followed within $\frac{1}{2}$ -2 days by ovulation and by pseudopregnancy, with an active corpus luteum of considerable duration. In the cat, cervical stimulation may probably be substituted for actual coitus (101). If no coitus occurs, the cat relapses into a quiescent state, to pass after a period of 2-3 weeks into another heat period. A considerable amount of work has been done with the rabbit, in determining the relationships between coitus, the release of gonadotropin from the pituitary gland, and ovulation. Fee and Parkes (81) showed that removal of the gland within an hour of copulation

prevents ovulation, but that delay in hypophysectomy beyond that time is ineffective in so doing. In the rabbit, ovulation occurs about 10½ hours after copulation, or after the injection of suitable gonadotropins (124). A summary of knowledge of the mechanism by which copulation causes the release of gonadotropin may be sought in Eckstein and Zuckerman (66).

It seems clear that these three cycle types depend primarily on the release of pituitary gonadotropins. The third rabbit-type cycle, completed only on demonstrable release of gonadotropin at copulation, is not in question. We are left to suppose, however, that the degree to which the corpus luteum is active after ovulation depends on the degree of prolactin secretion. Thus, in the first type of cycle, as in the rat, little or no prolactin is released unless copulation occurs, whereas in the second type this release regularly occurs. In natural circumstances, therefore, the three cycle types include three different ways of reacting to copulation. The normal release of LH (or LH and FSH) but of prolactin only at copulation produces a type 1 cycle; the release of both LH and prolactin with or without copulation produces a second type of cycle and the release of neither LH nor presumably of prolactin without copulation produces a third type of cycle. All this on the assumption that prolactin is luteotropin.

Type 2 cycles themselves show a considerable variation, indicating differences in the typical period during which the pituitary gland releases various types of gonadotropin and probably also in the sensitivity of the female tract and the possible development of refractoriness to continued estrogenic stimulation. Anterior pituitary FSH activity is highest in man, then come the horse, pig, sheep, and cattle in that order, which is the same order as the duration of the heat period. The threshold to estrogens is also highest in man and lowest in cattle, and so differences in the pituitary FSH output tend to be minimized to some extent, but not entirely. The very low threshold and the development of refractoriness in the cow is thought by some to be responsible for heat ceasing before ovulation—i.e., when estrogen is still high (7). The corpus luteum usually lasts for about 2 weeks, although it functions in some species for a much shorter time (2–3 days in the mare). The decline of the corpus luteum may overlap the growth of new follicles and so type 2 cycles may last from an average of about 17 days in the ewe to the 21 days more typical of other domestic species, such as the cow, mare, or pig.

VI. RELAXIN

A. *Nature and Distribution*

The biochemistry and physiology of relaxin have been the subject of two recent reviews (89, 127). Relaxin is a substance of protein or polypeptide nature, capable of relaxing the pelvic tissues of certain species after prior sensitization with estrogen. Relaxation in the primed guinea pig when given progesterone is due to the formation of relaxin in the reproductive tract itself. There also seems to be a number of related substances, probably very close to relaxin in constitution, which may copy one action or another of relaxin itself.

Hisaw and his colleagues have concentrated particularly on relaxin in the guinea pig, and in the most recent review quoted above they carefully define the relaxin they are discussing as the hormone producing effects in that species. It is possible to separate that activity, to some extent, from a similar activity in the mouse.

Relaxin has been found in the blood, placenta, and reproductive organs of mammals, and the ovary, uterus, and placenta have been suggested as sites of formation (125, 127). Castrated pregnant guinea pigs relax normally and show a normal blood concentration of relaxin (127, 180). The placenta also contains more relaxin than the uterus in the rabbit, suggesting the former as the source of hormone. On the other hand, Hall and Newton (105) believe that in the mouse the placenta acts by stimulating the ovary to produce relaxin. The ovary of the pregnant sow contains vast quantities of the hormone (3, 126) and there would seem to be little doubt as to its formation in the corpora lutea. There would thus seem to be species differences resembling others met with in endocrine research; in some mammals the ovary while in others the placenta appears to be the main site of relaxin production. Relaxin has been reported in tissues from the sow, guinea pig, rabbit, human, dog, cat, mare, and even the chicken.

B. *Physiological Action*

Relaxation of the human pelvic articulations in pregnancy and parturition was described by Snelling (231), and has been recorded by other authors in the guinea pig, mouse, dog, mole, cow, ewe, seal, and pocket gopher. Only the guinea pig and mouse have been studied in any detail.

Early studies with the guinea pig by Hisaw and his colleagues were considerably clouded by the fact that estrogen and combinations of estrogen and progesterone can cause pelvic relaxation. They showed, however, that the castrated guinea pig sensitized by prior estrogen treatment responds to relaxin preparations, and the studies were confirmed by

others (1, 25) Hisaw and his colleagues then showed that relaxin causes relaxation within 6 hours, while progesterone takes 72 to 96 hours and was only effective in animals with a uterus. Finally it was shown that progesterone can act by causing the formation of relaxin in the uterus, and that very potent relaxin extracts may have no steroid action.

Relaxation with estrogen in the guinea pig causes a resorption of bone, proliferation of loose fibrous tissue, that with relaxin causes a splitting of the collagenous fibers into thin threads and a breakdown of glycoproteins. Progesterone causes the same phenomena as does relaxin (127). In the mouse, treatment with estrogen causes pubic separation, with the formation of a gap 2-3 mm long, occupied by an interpubic ligament. Relaxin causes a separation more rapidly, which reaches something like twice the dimensions of that caused by estrogen. In the estrogenized mouse, progesterone does not cause pubic separation (104, 105).

Relaxin has other actions than relaxation, surveyed in the reviews quoted above. It appears to synergize with estrogen and progesterone in the rat in causing mammary growth, it has an antidiuretic action in the rabbit, it inhibits the deciduoma reaction of the rat uterus, and the rhythmic contractions of the uterus in guinea pigs and rats. It also seems to cause a pregnancy anemia in rabbits, an erythrocyte and hematocrit drop of about 33%, and a rise in reticulocytes.

C Assay of Relaxin

Hisaw and his associates prefer to assay relaxin by the manual palpation of castrated, estrogen primed guinea pigs 6 hours after injection. A "total response" is calculated as the product of the percentage of animals responding multiplied by the average degree of response, this is clearly the same as the total of all responses divided by the group number. The mouse test of Hall and Newton (103, 104) uses X ray photographs of the pubic bones, from which their separation can be measured. Talmage and Hurst (235) find an exponential relationship between dose and response using a similar technique in the guinea pig, while Dorfman *et al* (62) and Kluman *et al* (142) have performed reasonably precise tests with the mouse X ray technique.

REFERENCES

- 1 Abramson, D., Hurwitt E., and Lesnick, G., *Surg Gynecol Obstet* 65, 335 (1937)
- 2 Addison, W. H. F., *J Comp Neurol* 28, 441 (1917)
- 3 Albert, A., Morey, W. L., and Zarrow, M. A., *Endocrinology* 40, 370 (1947)
- 4 Allen, E., *Am J Anat* 30, 297 (1922)
- 4a Allen, E., in *Glandular Physiology and Therapy*, p. 145. American Medical Assoc., Chicago, Illinois, 1942.

5. Allen, E., and Doisy, E. A., *J. Am. Med. Assoc.* 81, 819 (1923).
6. Arenas, N., and Sammartino, R., *Bull. histol. Appl. et tech. microscop.* 16, 229 (1939).
7. Asdell, S. A., "Patterns of Mammalian Reproduction." Comstock, New York, 1948.
8. Astwood, E. B., *Endocrinology* 23, 25 (1938).
9. Bachman, C., Collip, J. B., and Selye, H., *Proc. Roy. Soc.* B117, 16 (1935).
10. Beach, F. A., "Hormones and Behaviour," p. 220. Hoeber, New York, 1948.
11. Bennetts, H. W., Underwood, E. J., and Shier, F. L., *Australian Vet. J.* 17, 85 (1948).
12. Bialek-Laprida, Z., *Compt. rend. soc. biol.* 114, 727, 733 (1933).
13. Biggers, J. D., *Nature* 170, 895 (1952).
14. Biggers, J. D., *J. Anat.* 87, 327 (1953).
15. Blair, A., *J. Exptl. Zool.* 103, 365 (1948).
16. Bloch, K., *Harvey Lectures* 48, 68 (1945).
17. Boeltinger, E. G., *J. Cellular Comp. Physiol.* 27, 9 (1947).
18. Bottomley, A. C., and Folley, S. J., *J. Physiol. (London)* 94, 28 (1938).
19. Brady, R. O., *J. Biol. Chem.* 193, 145 (1951).
20. Brambell, F. W. R., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), Vol. 1, Pt. 1, Chapt. 5. Longmans, Green, London, 1958.
21. Brambell, F. W. R., *Proc. Roy. Soc.* B103, 258 (1928).
22. Braunsberg, H., Osborn, S. B., and Stern, M. I., *J. Endocrinol.* 11, 177 (1954).
23. Breneman, W. R., *Endocrinology* 58, 262 (1958).
24. Brill, L., and Cuypers, Y., *Arch. intern. physiol.* 62, 70 (1954).
25. Brouha, L., and Simonnet, H., *Compt. rend. soc. biol.* 99, 1769 (1928).
26. Bulbring, E., and Burn, J. H., *J. Physiol. (London)* 85, 320 (1935).
27. Bullough, W. S., *Vitamins and Hormones* 13, 281 (1955).
28. Bullough, W. S., and van Oordt, G. J., *Acta Endocrinol.* 4, 241 (1950).
29. Burrows, H., "Biological Actions of Sex Hormones." Cambridge, Univ. Press. London and New York, 1945.
30. Burrows, H., *Brit. J. Surg.* 21, 507 (1934).
31. Butenandt, A., and Kudzusz, H., *Z. physiol. Chem.* 237, 75 (1935).
32. Butt, W. R., Morris, P., Morris, C. J. O. R., and Williams, D. C., *Biochem. J.* 49, 434 (1951).
33. Callow, N. H., Callow, R. K., and Emmens, C. W., *Biochem. J.* 32, 1312 (1938).
34. Camus, L., and Cley, E., *Compt. rend. soc. biol.* 48, 787 (1896).
35. Camus, L., and Cley, E., *Compt. rend. soc. biol.* 49, 787 (1897).
36. Casida, L. E., and McKenzie, F. F., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 170* (1932).
37. Catchpole, H. R., Hamilton, J. B., and Hubert, C. R., *J. Clin. Endocrinol.* 2, 181 (1942).
38. Chang, M. C., *J. Endocrinol.* 3, 192 (1942).
39. Christiansen, E. C., *Danish Med. Bull.* 3, 229 (1956).
40. Chu, J. P., and You, S. S., *Proc. Chinese Physiol. Soc. (Chengtu)* 2, 89 (1944).
41. Clayton, C. W., Bongiovanni, A. M., and Papadatos, C., *J. Clin. Endocrinol.* 15, 693 (1955).
- 41a. Clegg, M. T., and Cole, H. H., *J. Animal Sci.* 13, 108 (1954).
42. Cole, H. H., and Miller, R. F., *Am. J. Anat.* 57, 39 (1935).
43. Coste, F., Laurent, F., and Delabarre, F., *Compt. rend. soc. biol.* 145, 835 (1951).

- 44 Courrier, R, *Compt rend soc biol* 85, 939 (1921)
- 45 Courrier, R, and Potvin, R, *Compt rend soc biol* 94, 878 (1926)
- 46 Cowie, A T, Folley, S J, Malpress, F H, and Richardson, K C, *J Endocrinol* 8, 64 (1952)
- 47 Craig-Bennett, A, *Phil Trans Roy Soc London Ser B* 219, 197 (1931)
- 48 Cupps, P T, and Asdell, S A, *J Animol Sci* 3, 351 (1944)
- 49 Dantchakoff, V, *Compt rend soc biol* 122, 168, 1307 (1936)
- 50 Dantchakoff, V, *Compt rend soc biol* 124, 195 (1937)
- 51 Dempsey, E W, *Am J Physiol* 120, 126 (1937)
- 52 Diczfalusy, E, *Mem Soc Endocrinol No* 3, 56 (1955)
- 53 Diczfalusy, E, *Acto Endocrinol Suppl* 31, 11 (1957)
- 54 Diczfalusy, E, Magnusson, A M, Nilsson, L, and Westman, A, *Endocrinology* 69, 581 (1957)
- 55 Dodd, J M, *Mem Soc Endocrinol No* 4, 166 (1955)
- 56 Donahue, J K, *Endocrinology* 28, 519 (1941)
- 57 Dorfman, R I, *Endocrinology* 42, 7 (1948)
- 58 Dorfman, R I, in "Hormone Assay" (C W Emmens, ed), Chapt 13 Academic Press, New York, 1950
- 59 Dorfman, R I, *Vitamins and Hormones* 10, 331 (1952)
- 60 Dorfman, R I, *Physiol Revs* 34, 138 (1954)
- 61 Dorfman, R I, in 'The Hormones' (G Pincus and K V Thumann, eds), Vol 3, Chapt 12 Academic Press, New York, 1955
- 62 Dorfman, R I, Marsters, R W, and Dinerstein, J, *Endocrinology* 52, 204 (1953)
- 63 Dorfman, R I, and Shipley, R A, "Androgens" Wiley, New York, 1956
- 64 Dyke, H B Van, and Chen, G, *Am J Anat* 58, 473 (1936)
- 65 Ebling, F J, *J Endocrinol* 5, 297 (1948)
- 66 Eckstein, P, and Zuckerman, S, in "Marshall's Physiology of Reproduction" (A S Parkes, ed), Vol 1, Pt 1, Chapt 4 Longmans, Green, London, 1956
- 67 Eckstein, P, and Zuckerman, S, in 'Marshall's Physiology of Reproduction' (A S Parkes, ed), Vol 1, Pt 1, Chapt 6 Longmans, Green, London, 1956
- 68 Edgar, R D, *Biochem J* 54, 50 (1953)
- 69 Ellison, E T, and Wolfe, J M, *Endocrinology* 19, 160 (1935)
- 70 Emmens, C W, *J Physiol (London)* 93, 416 (1938)
- 71 Emmens, C W, *Med Research Council (Brit) Spec Rept Ser* 234 (1939).
- 72 Emmens, C W, *J Endocrinol* 2, 444 (1941)
- 73 Emmens, C W, 'Principles of Biological Assay' Chapman & Hall, London, 1948
- 74 Emmens, C W, in 'Hormone Assay' (C W Emmens, ed), p 391 Academic Press, New York, 1950
- 75 Emmens, C W, *Brit Med Bull* 11, 135 (1955)
- 76 Emmens, C W, and Bradshaw, T E T, *J Endocrinol* 1, 378 (1939)
- 77 Emmens, C W, and Parkes, A S, *J Endocrinol* 1, 323 (1939)
- 78 Emmens, C W, and Parkes, A S, *J Endocrinol* 1, 332 (1939)
- 79 Emmens, C W, and Parkes, A S, *Vitamins and Hormones* 5, 233 (1947)
- 80 Evans, H M, and Cole, H H, *Mem Unto Calif* 9, 66 (1931), quoted by Eckstein and Zuckerman (67)
- 81 Gee, A R, and Parkes, A S, *J Physiol (London)* 67, 383 (1929)
- 82 Fellner, O O, *Klin Wochschr* 4, 1651 (1925)
- 83 Fichera, C, *Arch ital biol* 43, 504 (1905)

84. Finkelstein, M., Hestrin, S., and Koch, W., *Proc. Soc. Exptl. Biol. Med.* **64**, 64 (1947).
85. Finney, D. J., "Statistical Method in Biological Assay." Charles Griffin, London, 1952.
86. Folley, S. J., "The Physiology and Biochemistry of Lactation." Oliver and Boyd, Edinburgh, 1958.
87. Forbes, T. R., *Am. J. Obstet. Gynecol.* **60**, 180 (1950).
88. Freud, J., *Biochem. J.* **27**, 1438 (1933).
89. Frieden, E. H., and Hisaw, F. L., *Recent Progr. in Hormone Research* **6**, 333 (1953).
90. Callagher, T. F., and Koch, F. C., *J. Pharmacol. Exptl. Therap.* **55**, 97 (1935).
91. Gallien, L., *Compt. rend.* **205**, 375 (1937).
- 91a. Callien, L., *Bull. biol. France et Belg.* **72**, 269 (1938).
- 91b. Callien, L., *Bull. biol. France et Belg.* **78**, 257 (1944).
- 91c. Callien, L., *Mem. Soc. Endocrinol.* **4**, 188 (1955).
92. Callien, L., *Compt. rend.* **231**, 919 (1950).
93. Cardner, W. U., *Proc. Soc. Exptl. Biol. Med.* **33**, 104 (1935).
94. Greenblatt, R. B., Mortara, F., and Torpin, R., *Am. J. Obstet. Gynecol.* **44**, 658 (1942).
95. Greene, R. R., and Burrill, M. W., *Endocrinology* **29**, 402 (1941).
96. Creene, R. R., Burrill, M. W., and Ivy, A. C., *Science* **88**, 130 (1938).
97. Creene, R. R., Burrill, M. W., and Ivy, A. C., *Am. J. Anat.* **65**, 415 (1939).
98. Creene, R. R., Burrill, M. W., and Ivy, A. C., *Am. J. Anat.* **67**, 305 (1940).
99. Creene, R. R., Burrill, M. W., and Ivy, A. C., *Physiol. Zool.* **15**, 1 (1942).
100. Greenwood, A. W., Blyth, J. S. S., and Callow, R. K., *Biochem. J.* **29**, 1400 (1935).
101. Crenlich, W. W., *Anat. Record* **56**, 217 (1934).
102. Grobstein, C., *Proc. Soc. Exptl. Biol. Med.* **49**, 477 (1942).
103. Hall, K., and Newton, W. H., *J. Physiol. (London)* **104**, 348 (1948).
104. Hall, K., and Newton, W. H., *Lancet* **i**, 54 (1946).
105. Hall, K., and Newton, W. H., *J. Physiol. (London)* **106**, 18 (1947).
106. Hamburger, C., *Acta Pathol. Microbiol. Scand. Suppl.* **17** (1933).
107. Hamilton, J. B., *Proc. Soc. Exptl. Biol. Med.* **35**, 386 (1936).
108. Hamilton, J. B., *Endocrinology* **21**, 649 (1937).
109. Hamilton, J. B., *J. Clin. Endocrinol.* **1**, 570 (1941).
110. Hamilton, J. B., *Recent Progr. in Hormone Research* **3**, 257 (1946).
111. Hammond, J., and Wodzicki, K., *Proc. Roy. Soc. B* **130**, 1 (1941).
112. Hardy, M. M., Biggers, J. D., and Claringbold, P. J., *Nature* **172**, 1196 (1953).
113. Harrison, R. J., *Biol. Revs. Cambridge Phil. Soc.* **23**, 290 (1946).
114. Hartman, C. G., *Yale J. Biol. and Med.* **17**, 99 (1944).
115. Haskins, A. L., *Proc. Soc. Exptl. Biol. Med.* **73**, 439 (1950).
116. Hatal, S., *Am. J. Anat.* **15**, 87 (1913).
117. Hatal, S., *J. Exptl. Zool.* **15**, 297 (1913).
118. Hazleton, L. W., and Goodrich, F. J., *J. Am. Pharm. Assoc. Sci. Ed.* **26**, 420 (1937).
119. Heape, W., *Quart. J. Microscop. Sci.* **44**, 1 (1900).
120. Heard, R. D. H., Bligh, E. C., Cann, M. G., Jellinek, P. H., O'Donnell, V. J., Rao, B. G., and Webb, J. L., *Recent Progr. in Hormone Research* **12**, 45 (1956).
121. Heard, R. D. H., and O'Donnell, V. J., *Endocrinology* **54**, 209 (1954).

- 122 Hensen, V, *Z Anat Entwicklungsgeschichte* 1, 213, 353 (1876)
- 123 Herlant, M, *Compt rend soc biol* 131, 1315, 1318 (1939)
- 124 Hill, M, and Parkes, A S, *J Physiol (London)* 71, 36 (1931)
- 125 Hisaw, F L, *Proc Soc Exptl Biol Med* 23, 661 (1926)
- 126 Hisaw, F L, and Zarrow, M X, *Proc Soc Exptl Biol Med* 69, 395 (1948)
- 127 Hisaw, F L, and Zarrow, F X, *Vitamins and Hormones* 8, 151 (1950)
- 128 Hoar, W S, *Mcm Soc Endocrinol No* 4, 5 (1955)
- 129 Hoffman, F, and Von Lam, L, *Zentr Gynakol* 76, 1177 (1948)
- 130 Hooker, C W, *Proc Soc Exptl Biol Med* 45, 270 (1940)
- 131 Horst, C J van der, and Gillman, J, *Anat Record* 80, 443 (1941)
- 132 Howard, E, *Am J Anat* 62, 381 (1938)
- 133 Howard, E, *Am J Anat* 65, 105 (1939)
- 134 Huggins, C, and Clark, P J, *J Exptl Med* 72, 747 (1940)
- 135 Huggins, C, and Hodges, C V, *Cancer Research* 1, 293 (1941)
- 136 Huggins, C, Masina, M H, Eichelberger, L, and Wharton, J D, *J Exptl Med* 76, 543 (1939)
- 137 Itho M, and Kon, T, *Compt rend soc biol* 120, 678 (1935)
- 138 Jongh, S E de, *Arch neerl physiol* 6, 28 (1935)
- 139 Jost, A, *Recent Progr in Hormone Research* 8, 379 (1953)
- 140 Kehl, R, and Combescot, C, *Mem Soc Endocrinol No* 4, 57 (1955)
- 141 Klein, M, and Parkes, A S, *Proc Roy Soc B* 121, 574 (1937)
- 142 Kluman, B, Salhanick, H A, and Zarrow, M X, *Endocrinology* 53, 391 (1953)
- 143 Kline, I T, and Dorfman, R I, *Endocrinology* 48, 345 (1951)
- 144 Kober, S, *Biochem Z* 239, 209 (1931)
- 145 Kochakian, C D, *Endocrinology* 21, 750 (1937)
- 146 Kochakian, C D, and Tillotson, C, *Endocrinology* 60, 607 (1957)
- 147 Korenchevsky, V, *J Pathol Bacteriol* 33, 607 (1930)
- 148 Korenchevsky, V, Dennison, M, and Kohn Speyer, A, *Biochem J* 26, 2097 (1932)
- 149 Korenchevsky, V, and Hall, K, *J Pathol Bacteriol* 45 687 (1937)
- 150 Korenchevsky, V, and Hall, K, *Brit Med J* 1, 4 (1939)
- 151 Korenchevsky, V, and Hall, K, *J Pathol Bacteriol* 50, 295 (1946)
- 152 Korenchevsky, V, Hall, K, Burburk, R C, and Ross, M A, *Biochem J* 33, 36 (1939)
- 153 Kraus, H, *J Physiol (London)* 61, 383 (1926)
- 154 Lacassagne, A, *Compt rend soc biol* 131, 586 (1933)
- 155 Lane, C E, and Hisaw, F L, *Anat Record* 60, 52 (1934)
- 156 Leonard, S L, *Endocrinology* 21, 330 (1937)
- 157 Loeb, L, *Proc Soc Exptl Biol Med* 4, 93 (1907)
- 158 Loeb, L, *Biol Bull* 27, 1 (1914)
- 159 Long J A, and Evans, H M, *Mcm Univ Calif* 6, 1 (1922)
- 160 Lyons, W R, and Templeton, H J, *Proc Soc Exptl Biol Med* 33, 587 (1936)
- 161 Makepeace, A W, Weinstein, G L, and Friedman, M H, *Proc Soc Exptl Biol Med* 35, 269 (1936)
- 162 Makepeace, A W, Weinstein, G L, and Friedman, M H, *Am J Physiol* 119, 512 (1937)
- 163 Mann, T, *Advances in Enzymol* 9, 329 (1949)
- 164 Mann, T, "The Biochemistry of Semen" Methuen London 1954

I. INTRODUCTION

Optimal reproductive activity would be expected to occur in animals when there is a proper balance of secretory activity of all the endocrine glands. Of greatest importance are the gonadotropic hormones of the pituitary and the hormones of the ovary and testes. Their optimal function can only occur in an otherwise normal animal. The present chapter is concerned with those hormones whose secretion rate may directly or indirectly influence the secretion rate of the above hormones or may so modify the animal metabolism that the normal functions of the above hormones are impaired.

Of these hormones, the role of thyroxine in reproduction has been most extensively studied. The great interest in thyroxine is due to its influence in energy metabolism at the cell level.

The hormones of the adrenal cortex are of vital importance to the survival of the animal. Whether they have specific roles in reproduction other than their general properties of maintenance of normal body function awaits further study.

The hormones of the parathyroid gland and pancreas also play important roles in maintaining normal body function, but more specific roles in reproduction are ill-defined. In a similar category is the growth hormone (somatotropin) of the anterior pituitary in relation to reproduction.

The role of relaxin in reproduction is of fundamental importance. Whether this hormone should be considered an ovarian hormone, in addition to estrogen and progesterone, or a hormone of pregnancy which is stimulated to secretion by the synergism of estrogen and progesterone in the reproductive tract is being investigated. That relaxin plays an important role in the maintenance of pregnancy and in the preparation of the birth canal for the expulsion of the fetus has been indicated.

The posterior pituitary hormone, oxytocin, has recently been shown to have three important functions in reproduction. First, it aids, in the rapid transport of semen toward the descending ovum following mating, second, it plays the role in the expulsion of the fetus at the time of parturition and, finally, causes the "let-down" of milk in the mammary gland.

The present chapter is primarily concerned with the role of the thyroid and adrenal glands in reproduction.

II. ROLE OF THYROID GLAND AND THYROXINE IN REPRODUCTION

The role of the thyroid gland and thyroxine in the reproductive process has been studied for many years. That thyroid gland function is important has been shown in many experiments, both by thyroidectomy and blockage of thyroxine secretion by the goitrogens to produce a hypothyroid state, and by the injection of thyroxine or the feeding of thyroprotein to produce mild hyperthyroidism.

While the general effects of hypo- and hyperfunction upon reproduction are generally recognized, the precise role of thyroxine in the process is more difficult to pinpoint. This is probably due to the very general effect of thyroxine upon the metabolism of the animal as well as the specific effects upon the secretory activity of many endocrine glands. Thyroxine might specifically influence the secretion of the gonadotropic hormones, it might influence the functional activity of the gonads in synergism with the gonadotropins, and it might influence the maintenance of pregnancy and lactation.

Maqsood (70) reviewed the literature concerning the role of the thyroid in relation to reproduction of mammals and birds.

In relating thyroxine secretion to reproductive efficiency, it is important to consider the effect of both hypo- and hypersecretion.

A. *Effects of Endemic Goiter*

Since thyroid hormone contains iodine, deficiencies of this element in the feed and water may cause deficiencies in thyroxine secretion rate and enlargement of the thyroid glands (goiter). Simple goiter has appeared in domestic animals, such as horses, cattle, sheep, goats, and pigs in many parts of the world where iodine deficiencies occur. In pigs, symptoms are hairlessness, big neck, stillbirth, or pigs weak at birth. In foals, deformation of joints and bones, stillbirth, or weakness occurs. In calves, the chief symptom is weakness or death at birth. In lambs and kids, big neck, weakness, or stillbirth occurs along with wool or hair deficiencies.

The general use of iodized salt in goitrous areas has corrected iodine deficiencies as a cause of thyroxine deficiencies; however, these observations are of interest in indicating reproductive problems related to thyroxine deficiencies.

B. *Experimental Hypo- and Hyperthyroidism*

By the technique of surgical thyroidectomy or graded levels of goitrogens, the influence of hypothyroidism upon the reproductive process may be studied satisfactorily. The problem of the study of

hyperthyroidism is far more complicated, however, and the variability of the results reported in respect to the effect on reproductive processes must be re-examined in the light of current knowledge of thyroid physiology. There are two fundamental factors influencing thyroxine secretion rate: (1) genetic-endocrine variability, and (2) environmental variability, of which ambient temperature and nutrition are most important.

How should hyperthyroidism be defined? Is it a level of thyroxine in excess of the mean secretion rate of a given population of animals, or in excess of the highest genetic potential secretion of individual animals? At elevated temperatures, by either definition, the degree of hyperthyroidism would change!

In the past, studies of hyperthyroidism were conducted without knowledge of the parameters of thyroid gland function in the species studied. In many of these experiments, the amount of thyroxine injected or the amount of desiccated thyroid or thyroprotein fed greatly exceeded any of these criteria of hyperthyroidism. Since overdoses of thyroxine, as with other stress effects, stimulates the secretion of the adrenocorticotrophic hormone (ACTH) and in turn the secretion of the adrenal cortical hormones, such experiments are not measures of hyperthyroidism but of the effect of stress-induced adrenal cortical hormone secretion on reproduction (123).

It is suggested that future studies of the effect of hyperthyroidism upon reproduction be related to the mean thyroxine secretion rate of the species or strains of animals being studied or to the highest genetic potential secretion rate. As an example, it was reported by Ward (124) that mice with a mean daily thyroxine secretion rate of 5.5 μg . thyroxine/100 g. body weight had normal conception and litter size when as much as four times the mean thyroxine secretion rate was administered. Levels of thyroxine above this range would be expected to gradually merge into a stress phenomenon.

Considerable data are now available concerning the average thyroxine secretion rate of experimental and domestic animals developed by the goitrogen technique (12, 13, 96), and by the recently developed methods employing radioactive iodine (14, 52, 81, 82, 83, 88). Dosage ranges of thyroxine in such studies can now be related to the mean secretion rate or to the range in secretion rate under controlled environmental conditions.

C. Variation in Thyroxine Secretion

Under uniform conditions of ambient temperature and nutrition, the thyroxine secretion rate of individual animals varies considerably

due to genetic-endocrine differences. In the case of dairy cattle the daily secretion rate of cows during the winter months varied from 2 mg. to 10 mg. thyroxine/1000 lb. body weight (85). When the secretion rates of high-producing cows are measured, it is quite probable that cows secreting as high as 15 mg. thyroxine/day or more will be found, since the cows included in the present study were relatively low producers. The mean daily thyroxine secretion rate was 5.6 mg./1000 lb. body weight. Considering a range of 2 mg. to 15 mg. thyroxine/day/1000 lb., the mean thyroxine secretion rate might be expected to be about 7 or 8 mg./day in a large population of dairy cattle.

Thyroprotein is an iodinated casein which contains 1% thyroxine. In our laboratory it has been found that the thyroxine contained is 10% as effective orally in cattle as when thyroxine is injected subcutaneously. Thus, 1 mg. of thyroxine injected has the same biological activity as 1 g. of thyroprotein when fed. It is recommended that dairy cattle be fed 15 g. of thyroprotein/day/1000 lb. body weight. It will be seen that this amount of thyroprotein equals the estimated thyroxine secretion of high-producing cows. It is between two and three times the average thyroxine secretion of cattle. When this amount of thyroprotein is fed, it brings up the effective level of circulating thyroxine to the level of those cows which have received an inheritance for optimal amounts of thyroxine secretion. When thyroprotein is fed in an amount equal to the cow's thyroxine secretion rate, endogenous thyroxine secretion is depressed so that the cow is totally dependent upon the thyroxine contained in the thyroprotein.

The presence of cows with low inherited thyroxine secretion rates raises the question as to their reproductive efficiency. May not the low thyroxine secretion rate influence their capacity to conceive? Some evidence of this influence will be presented later. The method of determining thyroxine secretion rate provides a fine tool for the study of this problem. It may be possible to show that cows with low inherited thyroxine secretion conceive with greater difficulty.

D. Seasonal Variation in Thyroxine Secretion

Indirect evidence has been presented over the years indicating that thyroid function is depressed as ambient temperature is increased. In a cold environment, with adequate nutritional conditions, the full potential inheritance for thyroxine secretion was thought to be expressed. As the ambient temperature increased, the secretion of thyroxine was believed to be depressed gradually. With the development of improved methods of determining thyroid function, the influence of ambient or

seasonal changes upon thyroxine secretion have been determined. Reineke and Turner (91) showed that the average thyroxine secretion of young chicks during the summer was only one-half that of the winter. Henneman *et al.* (52) recently reported a 4-fold reduction in the thyroxine secretion rate of sheep during the summer as contrasted with the winter, and Premachandra *et al.* (84) reported a 3-fold reduction in

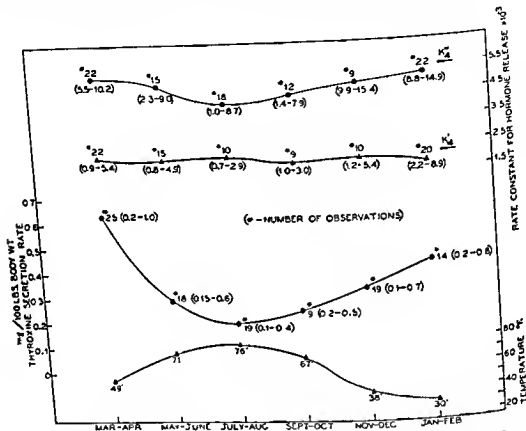


FIG. 1. The inverse relation between thyroxine secretion rate of dairy cattle and changing seasonal ambient temperature is shown. In contrast, the normal thyroidal- ^{131}I release rate (K') shows little seasonal variation. When recycling of iodine is controlled with thiouracil, the release rate (K'') shows a slight seasonal decline in summer.

heifers and cows during the summer months. Under Missouri climatic conditions, a regular cycle of thyroxine secretion rate in relation to ambient temperature changes was observed (Figs. 1 and 2).

The seasonal changes in thyroxine secretion rate, especially the marked reduction during the summer months, would tend to influence the reproductive process to the extent that hypothyroidism affects reproduction.

With this introduction, let us now turn to a review of the literature

concerned with the more specific effects of hypo- and hyperthyroidism upon the reproductive process.

E. Pituitary Gonadotropins

Reports of the influence of hypothyroidism upon the secretion of the gonadotropic hormones has been variable. Although some contradictory reports exist in the earlier literature (89) and differentiation of the effect upon FSH and LH was not studied, the majority of the investiga-

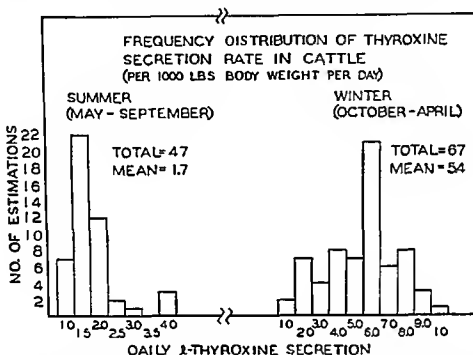


FIG. 2. The individual estimations of thyroxine secretion rates of cattle indicate a 10-fold variation in winter. This variation is believed to reflect primarily genetic differences in thyroid function. The 4-fold variation in thyroxine secretion rate in summer reflects the important influence of ambient temperature on thyroid function.

tors reported reduced concentration of these hormones in thyroidectomized or goitrogen-treated animals. Atalla and Reineke (6) fed mice 0.1% thiouracil or 0.005 to 0.2% thyroprotein. All groups fed thyroprotein showed earlier opening of the vagina and onset of estrous cycles. They concluded that hyperthyroid mice secrete increased luteinizing hormone (LH), while in hypothyroidism follicle-stimulating hormone predominates. Indirect evidence concerning the reduced sexual activity in both male and female cattle would support the thesis of reduced LH secretion.

F. Response of Gonadotropins

A number of studies have been reported concerning the effect of hypo- and hyperthyroidism upon the response of gonadotropins in immature animals. The earlier studies are reviewed by Meites and Chand-

seasonal changes upon thyroxine secretion have been determined. Reineke and Turner (91) showed that the average thyroxine secretion of young chicks during the summer was only one-half that of the winter. Henneman *et al.* (52) recently reported a 4-fold reduction in the thyroxine secretion rate of sheep during the summer as contrasted with the winter, and Premachandra *et al.* (84) reported a 3-fold reduction in

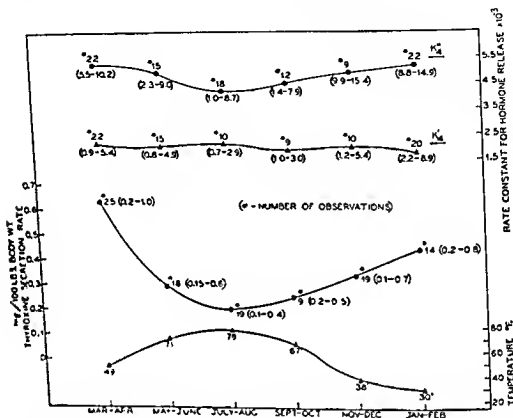


FIG. 1. The inverse relation between thyroxine secretion rate of dairy cattle and changing seasonal ambient temperature is shown. In contrast, the normal thyroidal- (121) release rate (K_4) shows little seasonal variation. When recycling of iodine is controlled with thiouracil, the release rate (K_4) shows a slight seasonal decline in summer.

heifers and cows during the summer months. Under Missouri climatic conditions, a regular cycle of thyroxine secretion rate in relation to ambient temperature changes was observed (Figs. 1 and 2).

The seasonal changes in thyroxine secretion rate, especially the marked reduction during the summer months, would tend to influence the reproductive process to the extent that hypothyroidism affects reproduction.

With this introduction, let us now turn to a review of the literature

concerned with the more specific effects of hypo- and hyperthyroidism upon the reproductive process.

E. Pituitary Gonadotropins

Reports of the influence of hypothyroidism upon the secretion of the gonadotropic hormones has been variable. Although some contradictory reports exist in the earlier literature (89) and differentiation of the effect upon FSH and LH was not studied, the majority of the investiga-

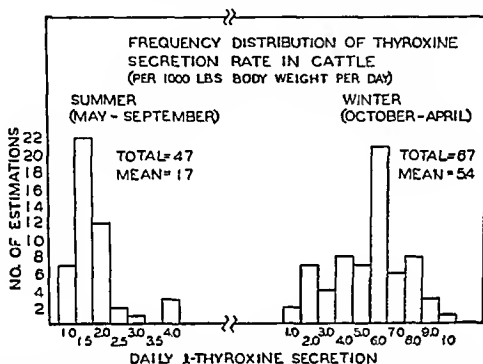


FIG. 2. The individual estimations of thyroxine secretion rates of cattle indicate a 10-fold variation in winter. This variation is believed to reflect primarily genetic differences in thyroid function. The 4-fold variation in thyroxine secretion rate in summer reflects the important influence of ambient temperature on thyroid function.

tors reported reduced concentration of these hormones in thyroidectomized or goitrogen-treated animals. Atalla and Reineke (6) fed mice 0.1% thiouracil or 0.005 to 0.2% thyroprotein. All groups fed thyroprotein showed earlier opening of the vagina and onset of estrous cycles. They concluded that hyperthyroid mice secrete increased luteinizing hormone (LH), while in hypothyroidism follicle-stimulating hormone predominates. Indirect evidence concerning the reduced sexual activity in both male and female cattle would support the thesis of reduced LH secretion.

F. Response of Gonadotropins

A number of studies have been reported concerning the effect of hypo- and hyperthyroidism upon the response of gonadotropins in immature animals. The earlier studies are reviewed by Meites and Chand-

rashaker (74) in reference to male gonad response. A constant amount of equine gonadotropin was administered to immature male rats in which a hypo-state was induced by thiouracil and a hyper-state by graded amounts of thyroprotein. The normal response of the seminal vesicles and coagulating glands was partially to completely inhibited by thyroprotein, whereas thiouracil stimulated increased response. On the other hand, thiouracil decreased the response while thyroprotein increased the response in mice. It was suggested that rats normally secrete more than an optimal amount of thyroxine, whereas mice secrete less than an optimal amount.

A similar species difference in response to equine gonadotropin in female rats and mice was observed by Johnson and Meites (62). Janes (61) however, used pituitary gonadotropin in hypothyroid female rats. He reported that short thiouracil treatment diminished the ovarian response, whereas, if of long duration, the response to gonadotropin was exaggerated as compared with that in control animals, both with large and small doses of gonadotropin. Histological examination of the large ovaries showed that the weight increase was caused chiefly by large corpora lutea and ovarian cysts. The ovaries of rats which were maintained on thiouracil for 70 days remained immature. Warner and Meyer (125) reported that thyroxine given to intact female rats in parabiotic union with ovariectomized females caused inhibition of ovarian hypertrophy. It was suggested that thyroxine reduced the sensitivity of the ovary to endogenous gonadotropin.

That the above generalization in regard to differences in rats and mice with respect to their thyroid status may apply as well to strain differences within species is shown by Stob and Andrews (104). Strains of mice were selected for large, medium, and small body size for five generations. It was shown that feeding 0.22% of thyroprotein significantly increased the growth rate in the medium- and small-sized groups but decreased the growth rate of the large mice. These results are interpreted as indicating lowered normal thyroxine secretion in the strains of small mice. Thiouracil fed at a level of 0.15% consistently reduced body weight in all lines but at the 0.05% level depressed growth only in the large line. Thyroprotein or thiouracil produced no significant alteration of gonad weight in either sex. The effect of gonadotropins upon the gonads of these mice was not studied.

G. Female Reproduction

Since Reinecke and Solomon (89) have reviewed the literature concerning the role of the thyroid hormone in the reproductive physiology of the female, it is only necessary here to summarize the observations

on experimental animals and add more details concerning the domestic animals.

In many species, aberrations of the estrous cycles have appeared in both hypo- and hyperthyroid animals. These effects may appear as lengthening, irregularity, or complete disappearance of the cycles.

In the bovine, thyroidectomy caused heifers to show an absence of the usual signs of heat (24, 101). However, it was observed by the manual palpation of the ovaries that ovulation occurred at regular intervals. Artificial insemination with semen from a thyroidectomized bull at the time of ovulation resulted in the birth of three normal offspring. The condition of "silent" estrus in these animals would indicate a mild deficiency in the secretion of the gonadotropins, especially LH, or of the gonadal hormones (estrogen or progesterone), or both. When replacement therapy was practiced, normal estrous cycles were resumed.

Engle (35) reported that the hypothyroid monkey is amenorrheic to the extent of about one or two periods a year. A hypothyroid monkey put on a very low thyroid dosage for a period of 10 days always resulted in the return of menstrual function. A single treatment will give either two or three successive normal menstrual cycles. In the hypothyroid monkey the amount of estrogen needed to induce estrogen-withdrawal bleeding is greatly increased. In monkeys treated with thiouracil the menstrual cycles become very irregular. Amenorrhea or extremely prolonged cycles of over 50 days were the rule. Engle stated, "I suspect that thyroid empirically given in most cases of menstrual disturbances in women gives a greater degree of success than any other hormone which is used."

The ovarian picture in hypothyroidism appears quite variable. In cattle, sheep, and goats, apparently normal follicle development and ovulation can occur. In experimental mammals, abnormal changes in the ovary may occur with marked depression of conception rate.

Thyroprotein has been fed extensively to lactating dairy cattle to stimulate increased milk production. There have been a number of incidental observations concerning its value in connection with reproductive problems, but no systematic study of the value of thyroprotein in cows of low fertility has been reported. At the recommended levels of feeding thyroprotein to dairy cattle, no undesirable effects upon reproduction have been reported. Cows pregnant when thyroprotein feeding was initiated have calved normally. Leach and Bailey (66) in long-time trials in England reported that the thyroprotein-fed group showed a reduced incidence of "abnormal" calvings. Thomas and Moore (107) reported an incidence of 6% stillbirths in first-calf heifers but none from cows fed thyroprotein.

Reece (86) reported on 2 cows being fed thyroprotein. One ewe had been bred 3 times prior to thyroprotein-feeding and had not conceived. She conceived on the fifth service during thyroprotein-feeding. A second cow had failed to conceive following 10 services before thyroprotein, but conceived on the third service after thyroprotein-feeding was started.

Thomas and Moore (107) reported that cows fed thyroprotein during lactation until 90 days before parturition produced normal-appearing calves without calving difficulties. However, the mortality rate was higher than normal and it was implied that the feeding of thyroprotein was responsible. No similar report of other investigators has been made, although thousands of cows have been fed thyroprotein. Since thyroprotein was withdrawn 90 days before parturition and normal endogenous thyroxine secretion returns within 30 days after withdrawal, there are no apparent physiological reasons for suspecting that the calf mortality observed is related to thyroprotein feeding; rather, it is suggested that poor calf management was involved.

H. Pregnancy

From the observations reported in the literature, it would appear that thyroidectomy of pregnant animals neither causes abortion nor prevents conception in sheep, goats, or cattle. Thus, Simpson (100) reported that no abortions occurred in thyroidectomized sheep. Reineke and Turner (90) reported that one thyroidectomized kid became pregnant, apparently to the service of a thyroidectomized male. This animal showed a marked increase in growth rate after about 10 weeks of pregnancy, apparently due to the placental transfer of fetal thyroid hormone. Spielman *et al.* (101) reported thyroidectomy of a heifer on her 46th day of gestation. Her growth was static for a period of about 20 weeks, then a sudden resumption of growth occurred similar to that reported in the goat. Three thyroidectomized helpers conceived and gave birth to normal calves.

I. Male Reproduction

Berliner and Warbritton (11) reported that thyroidectomy of rams produced a decrease in semen volume, spermatozoan concentration, and an increase in the relative numbers of abnormal sperm. Thyroxine administered to the thyroidectomized ram and to others of lowered fertility resulted in the production of semen with an increased number of sperm and decreased abnormal sperm.

Thyroxine and thyroprotein were reported (20) to alleviate symptoms of "summer sterility" resulting from impaired spermatogenic activ-

ity. The feeding of thiouracil during the fall breeding season maintained semen characteristics of the summer months. It was concluded that the thyroid gland is of major importance in the reproductive physiology of the ram.

The effect of thyroidectomy upon a 4-month-old Jersey bull calf was reported (80). While the gonads appeared to develop normally, there was complete absence of libido at sexual maturity when tested with cows in heat. By rectal palpation ejaculates were obtained which were normal in amount and sperm number. Semen samples so obtained were used in artificial insemination of cows and pregnancies resulted.

Reineke (87) reported upon the effects of feeding thyroprotein to a group of dairy bulls averaging about 8 years of age which had become rather sluggish and lethargic and showed reduced sex drive. In these animals thyroprotein feeding rather consistently caused an improvement in sexual drive and vigor. The limited conception records suggested an improvement in spermatogenesis. The time required for an observable effect to occur ranged from 7 to 40 days, and averaged 16 days.

In the young male mouse kept at 24°C., the administration of moderate amounts of thyroprotein increased the testes and seminal vesicle weights, and increased spermatogenic activity. Larger doses adversely affected the development of the testes. At 30°C., the control testes and seminal vesicles were lighter due to reduced thyroxine secretion. The lower levels of thyroprotein stimulated the testes as at the lower temperature, but the animals showed less response at the higher dosages. Thiouracil depressed the growth and functional activity of the testes (72).

In the adult male guinea pig the strength of sex drive was not altered significantly by thyroidectomy or by daily injections of thyroxine (137). However, it was suggested that hypothyroidism lowers the percentage of fertile matings (136).

In the male rabbit, Maqsood (71) reported continuous feeding of thyroprotein (between 0.004 and 0.008% of the ration) within optimal physiological limits resulted in precocious sexual maturity when compared with controls, whereas large doses adversely affected development and function. Doses below or about equal to the estimated thyroxine secretion rate had no effect. The feeding of 0.1% thiouracil in the ration of male rabbits for short periods resulted in significant decreases in testes weights and spermatogenic activity. Prolonged thiouracil treatment or thyroidectomy arrested the onset of sexual maturity, and animals under either treatment expressed neither libido nor ejaculated.

Lack of sexual desire indicated reduced male sex hormone production. Similar results were reported in male sheep.

J. Fertility of Rams in Summer

To reduce the effect of high ambient temperature on the thyroxine secretion rate of Southdown rams, Dutt and Simpson (34) kept one group in an air-conditioned room at 45–48°F. and compared them with a group of rams kept at environmental temperatures (Kentucky). No difference in semen volume was observed, but from August 20 to September 24 the average motility ratings for weekly collections was 70.3% for cooled rams and 41.8% for control rams, and 6.4% abnormal sperm in contrast to 36.9%. The cooled rams had a higher sperm cell concentration (3.4 vs. 2.4 million cells per μ l.). Embryonic death loss was higher in ewes bred to control rams. These results indicate the beneficial effect of low temperature in maintaining higher reproductive performance.

K. Hyperthyroidism in Birds (Male)

Crew (28) reported that the feeding of desiccated thyroid to 5- to 8-year-old cocks and hens promptly caused molt, then stimulated egg production at a faster rate; the head furnishings became red and turgid. After six months treatment was discontinued and the birds became progressively senile again.

Jaap (59) studied the effect of thyroid feeding upon testis size and spermatogenesis of Mallard drakes during late winter and early spring. The response was related to the amount of thyroid fed (0.25–1.0 g.) with testes ranging from 2 to 10 times the weight of the controls. The largest testes showed the presence of large numbers of spermatids. Aron and Benoit (3) found that feeding thyroid caused sexual stimulation in immature drakes.

A young, thyroidectomized, Brown Leghorn cockerel was fed 20 mg. daily of dried thyroid substance by Greenwood and Chu (43) for a period of 80 days. During this time the comb of the bird increased in size by 72 mm., while untreated birds showed an average increase in comb size of 8 mm. Following the cessation of thyroid treatment the comb underwent regression, decreasing in size by 26 mm. during the succeeding 80 days.

Titus and Burrows (108) fed 6-month-old White Leghorn cockerels 100 mg. of desiccated thyroid three times per week, starting in May for a period of 5 weeks. The seasonal decline in semen volume was increased by this treatment. When treatment was stopped, there was a temporary

increase in semen volume. It would appear from these results that this dosage of thyroid was excessive.

Turner *et al.* (111) fed Barred Plymouth Rock cockerels for 12 weeks on a ration containing 0.1% (45 g./100 lb. feed) thyroprotein. The normal growth of the testes was reduced, especially at 10 and 12 weeks, the final average testis weight being only 332.5 mg. as compared to 608.9 mg. for the controls. Wheeler *et al.* (127) reported a highly significant decrease in weight of the testis and a significant decrease in weight of the comb of 12-week-old Rhode Island Red cockerels fed thyroprotein at the level of 10 g./100 lb. feed from hatching time. When similar birds were carried to 24 weeks of age, body weight was significantly greater on thyroprotein feeding. Both relative and absolute testis weights were significantly greater (controls, 16.25 g.; thyroprotein-fed, 27.27 g.).

Glazener and Jull (40) fed Barred Plymouth Rock \times New Hampshire chicks 0.1% desiccated thyroid beginning the 4th week and continued until the 10th week. The average testis weight was 420 mg. compared to 1356 mg. for the controls. The comb size was also reduced 3.66 cm. as compared to 10.54 cm. (length \times width).

The feeding of 1 mg. tablets of thyroxine to each of 4 Rhode Island Red cocks three times weekly from January 8 to March 14 was reported by Hays (49). The hormone treatment produced no significant effect on the fertility of either young or old males in natural matings.

Martinez-Campos (73) observed no significant change in semen production or in spermatogenesis in Rhode Island Red cockerels during the feeding of 0.01% and 0.02% of thyroprotein in the ration for a period of 3 weeks. The feeding of 0.04% in the ration, however, caused an increase in the semen volume, sperm concentration, and total number of sperm per ejaculate. Wilwerth (130) extended and confirmed the above observations with the same breed of cockerels. Thyroprotein as 0.04% (18 g./100 lb. feed) of the ration caused an increase above normal controls in semen volume and a significant increase (5% level) in sperm concentration. The total number of sperm per ejaculate increased proportionately. The feeding of 0.08% thyroprotein caused a highly significant decrease in semen volume and sperm concentration, which was decreased still further when the dosage level was increased to 0.16%.

The feeding of thyroprotein to Barred Rock cockerels at the level of 10 g. per 100 lb. of feed has been reported by Shaffner (98). Feeding was started in April and was continued for 16 weeks. Data on the fertility of the semen after 12 and 15 weeks of treatment were obtained. A statistical analysis of the fertility data of individual males showed that

Lack of sexual desire indicated reduced male sex hormone production. Similar results were reported in male sheep.

J. Fertility of Rams in Summer

To reduce the effect of high ambient temperature on the thyroxine secretion rate of Southdown rams, Dutt and Simpson (34) kept one group in an air-conditioned room at 45-48°F. and compared them with a group of rams kept at environmental temperatures (Kentucky). No difference in semen volume was observed, but from August 20 to September 24 the average motility ratings for weekly collections was 70.3% for cooled rams and 41.8% for control rams, and 6.4% abnormal sperm in contrast to 36.9%. The cooled rams had a higher sperm cell concentration (3.4 vs. 2.4 million cells per μ l.). Embryonic death loss was higher in ewes bred to control rams. These results indicate the beneficial effect of low temperature in maintaining higher reproductive performance.

K. Hyperthyroidism in Birds (Male)

Crew (28) reported that the feeding of desiccated thyroid to 5- to 8-year-old cocks and hens promptly caused molt, then stimulated egg production at a faster rate; the head furnishings became red and turgid. After six months treatment was discontinued and the birds became progressively senile again.

Jaap (59) studied the effect of thyroid feeding upon testis size and spermatogenesis of Mallard drakes during late winter and early spring. The response was related to the amount of thyroid fed (0.25-1.0 g.) with testes ranging from 2 to 10 times the weight of the controls. The largest testes showed the presence of large numbers of spermatids. Aron and Benoit (3) found that feeding thyroid caused sexual stimulation in immature drakes.

A young, thyroidectomized, Brown Leghorn cockerel was fed 20 mg. daily of dried thyroid substance by Greenwood and Chu (43) for a period of 80 days. During this time the comb of the bird increased in size by 72 mm., while untreated birds showed an average increase in comb size of 8 mm. Following the cessation of thyroid treatment the comb underwent regression, decreasing in size by 26 mm. during the succeeding 80 days.

Titus and Burrows (108) fed 8-month-old White Leghorn cockerels 100 mg. of desiccated thyroid three times per week, starting in May for a period of 5 weeks. The seasonal decline in semen volume was increased by this treatment. When treatment was stopped, there was a temporary

increase in semen volume. It would appear from these results that this dosage of thyroid was excessive.

Turner *et al.* (111) fed Barred Plymouth Rock cockerels for 12 weeks on a ration containing 0.1% (45 g./100 lb. feed) thyroprotein. The normal growth of the testes was reduced, especially at 10 and 12 weeks, the final average testis weight being only 332.5 mg. as compared to 608.9 mg. for the controls. Wheeler *et al.* (127) reported a highly significant decrease in weight of the testis and a significant decrease in weight of the comb of 12-week-old Rhode Island Red cockerels fed thyroprotein at the level of 10 g./100 lb. feed from hatching time. When similar birds were carried to 24 weeks of age, body weight was significantly greater on thyroprotein feeding. Both relative and absolute testis weights were significantly greater (controls, 16.25 g.; thyroprotein-fed, 27.27 g.).

Glazener and Jull (40) fed Barred Plymouth Rock \times New Hampshire chicks 0.1% desiccated thyroid beginning the 4th week and continued until the 10th week. The average testis weight was 420 mg. compared to 1356 mg. for the controls. The comb size was also reduced 3.66 cm. as compared to 10.54 cm. (length \times width).

The feeding of 1 mg. tablets of thyroxine to each of 4 Rhode Island Red cocks three times weekly from January 8 to March 14 was reported by Hays (49). The hormone treatment produced no significant effect on the fertility of either young or old males in natural matings.

Martinez-Campos (73) observed no significant change in semen production or in spermatogenesis in Rhode Island Red cockerels during the feeding of 0.01% and 0.02% of thyroprotein in the ration for a period of 3 weeks. The feeding of 0.04% in the ration, however, caused an increase in the semen volume, sperm concentration, and total number of sperm per ejaculate. Wilwerth (130) extended and confirmed the above observations with the same breed of cockerels. Thyroprotein at 0.04% (18 g./100 lb. feed) of the ration caused an increase above normal controls in semen volume and a significant increase (5% level) in sperm concentration. The total number of sperm per ejaculate increased proportionately. The feeding of 0.08% thyroprotein caused a highly significant decrease in semen volume and sperm concentration, which was decreased still further when the dosage level was increased to 0.16%.

The feeding of thyroprotein to Barred Rock cockerels at the level of 10 g. per 100 lb. of feed has been reported by Shaffner (98). Feeding was started in April and was continued for 16 weeks. Data on the fertility of the semen after 12 and 15 weeks of treatment were obtained. A statistical analysis of the fertility data of individual males showed that

thyroprotein feeding at this level resulted in a significant reduction in fertility.

Kumaran and Turner (65) induced mild hyperthyroidism in White Plymouth Rock cockerels by feeding thyroprotein at levels of 0.04 and 0.08% of the ration to 14 weeks of age. At the 0.04% level there was slightly faster growth in the 8- and 10-week-old groups but the 12- and 14-week-old groups were about the same as the controls. While spermatogenesis proceeded at approximately a normal rate, the average comb weight of each group throughout the experiment was markedly increased over their control group, indicating that the rate of secretion of male hormone was increased. At the 0.08% thyroprotein level slight stimulation of spermatogenesis was observed with marked stimulation of comb growth.

Fredcen (37) fed White Wyandotte and New Hampshire cockerels 10 g. thyroprotein per 100 lb. feed at the start of the breeding season. Reproductive performance was measured as the duration of fertility resulting from a single insemination with a standard quantity of semen. Insemination from the thyroprotein-fed males gave the greatest duration of fertility and was most marked in the White Wyandotte breed. An additional trial, repeated 3 times in each of two years in which thyroprotein was fed continuously from the time of hatching, indicated reproductive performance of the males was increased in both breeds.

Wilwerth *et al.* (131) fed Rhode Island Red cocks on 0.01, 0.02, and 0.04% thyroprotein. It caused a significant increase in semen volume, the greatest effect being observed at the highest level. In a second trial, 0.01% was compared with 0.03 and 0.16%. The 0.04% level increased sperm concentration with no effect on semen volume, but the higher levels significantly decreased both semen volume and concentration.

L. Hyperthyroidism in Birds (Female)

Booker and Sturkie (21) demonstrated that hens laying four eggs in sequence showed a higher thyroid function than similar hens laying two eggs in sequence. Ten grams of thyroprotein per 100 lb. of feed was found to be near the optimum dosage for yearling White Leghorn hens to maintain egg production, and higher dosage levels resulted in weight losses and cessation of egg production (112). Many investigators have used this dosage level in testing the effects of thyroprotein on egg production and reported controversial results. An increase in egg production from feeding thyroprotein over a period of time, up to 7 years, has been observed (112, 113, 114, 115, 116) in aged White Leghorn hens, and throughout the entire laying year of White Plymouth

Rock pullets (110). Experiments indicated that the principal effect of thyroprotein feeding was the maintenance of egg production during periods of decline in basal metabolism caused by high summer temperatures (23, 133) and increasing age (110). Oloufa (78) fed thyroprotein at a level of 5 g. per 100 lb. of feed to Egyptian hens during summer months. The result indicated a significant increase in egg production.

Other investigators feeding 10 g. of thyroprotein per 100 lb. of feed to laying hens have observed no improvement in egg production (41, 44, 45, 55, 57, 67, 77, 95, 129). A few reports indicate that the above level of treatment may affect egg production adversely (55, 57, 77). Wilson (130) studied the effects of thyroprotein feeding on laying hens maintained under controlled high temperature environment. Berg and Bearse (10) reported decreased egg production in caged White Leghorn pullets fed thyroprotein (15 g. per 100 lb. feed) for a period of 10 weeks.

Effects of hyperthyroidism produced by feeding thyroprotein to laying hens may be noted in decrease of body weight (10, 15, 55); it was also observed in males (98). Decrease in thyroid weight was observed by Turner *et al.* (113) and Turner (109), and in the lengthening of the incubation time required for normal hatching by Wheeler and Hoffman (128).

M. Effect of Hyperthyroidism on Egg Quality

Asmundson (4) and Asmundson and Pinsky (5) demonstrated that feeding desiccated thyroid to laying hens resulted in egg shells that were significantly heavier than those from the controls. A significant improvement in egg shell thickness has been observed consistently in thyroprotein-fed laying hens (10, 38, 41, 45, 55, 67, 77, 95, 129).

No significant difference in egg weight has been observed in thyroprotein-fed laying hens (10, 38, 41, 55, 57, 78, 95).

N. Possible Causes of the Diversity of Results in Induced Hyperthyroidism

The possible causes of diversity in the observations with regard to induced hyperthyroidism in male and female birds should be discussed. The first factor involved is the biological activity of the hormonal preparations. In the use of desiccated thyroid and the early preparations of thyroprotein, the products were not standardized biologically. At the present time, thyroprotein is standardized to contain 1% thyroxine. The oral effectiveness of thyroprotein in relation to thyroxine by injection still requires further study. Preliminary evidence indicates that it is more effective orally than the 10% observed in ruminant animals.

The second problem that requires much further study is the normal

variation in thyroxine secretion in breeds and varieties of birds. While progress in this area of study has progressed as a result of the introduction of the goitrogen technique (96) and later by the study of radioactive iodine of the blood (14), only recently has a method been developed which satisfactorily measures the thyroxine secretion rate of individual birds (83). Using this method, wide variation in secretion rate of two strains of birds has been observed (85a). It will now be possible to study the changes in thyroxine secretion rate of birds in relation to growth and aging, seasonal variation, egg production, etc. Knowing the normal range in secretion rate in breeds of birds, it will be possible intelligently to provide physiological levels of thyroprotein to bring up the effective level of thyroxine in the blood to the optimum.

Breeds and strains of birds which have received an inheritance for optimum thyroxine secretion rate cannot be expected to respond to thyroprotein feeding.

Seasonal variation in thyroxine secretion rate has been suggested as a cause of reduced egg production in the summer months and the reason for the effectiveness of thyroprotein feeding at this time. It should be pointed out that in the northern United States and Canada, and other places with cool summers, the seasonal variation in thyroxine secretion rate may not be great and less benefit would result from the feeding of thyroprotein in those regions.

In general, mild hyperthyroidism can only be expected to be effective in improving reproductive performance in birds with lower genetic thyroxine secretion rates or when influenced by ambient temperature or aging.

O. Hypothyroidism in Birds (Males)

Benoit and Aron (9) reported that the testes of White Leghorn cockerels after thyroidectomy decreased as much as 80% in 11 days and 90% in 20 days. Similar results were obtained with drakes. Thyroidectomy reduced considerably the testicular growth in immature ducks exposed to the stimulating action of electric light for 15 hours per day during three weeks (7). The inhibition of the testes continued for about 34 days, after which the inhibition diminished to the 60th day (8). The development of the penis was inhibited more than the testis weight (8a).

Greenwood and Chu (43) observed regression in testicular size and cessation of spermatogenesis in Brown Leghorns after thyroidectomy. Blivaiss and Domm (19) thyroidectomized cockerels from the 4th to 20th days after hatching. Their adult weight was 40-45% below that of normal, their head furnishings were smaller, the combs were smaller, and the

less than those of controls. At 8 months of age the testes had smaller tubules and in no case showed development stages beyond spermatocytes.

Payne (79) reported the gonad and comb weights of thyroidectomized White Leghorn chicks were smaller even in comparison to body size.

Schultze and Turner (96) noted a significant decrease in testes weight when White Leghorn cockerels were fed 0.1% thiouracil in the feed from the 10th to 12th weeks, but not when fed from the 12th to 15th weeks.

Andrews and Schnetzler (1) fed cockerels, from 6 to 14 weeks, a ration containing 0.1 and 0.2% thiouracil. There was a progressive decrease in testis and comb weight as the amount increased. These observations were confirmed by Jull (63) and Japp (60).

Shaffner and Andrews (99) fed sexually mature cocks for 18 weeks on rations containing 0.2 and 0.5% thiouracil. There was a significant decrease in the average testis weight, diameter of the seminiferous tubules, and average comb area in comparison to the controls.

Both Martinez-Campos (73) and Shaffner and Andrews (99) reported that thiouracil did not influence sperm concentration, semen volume, or motility, but it may have influenced survival time in the oviduct.

Kumaran and Turner (65) fed a ration containing 0.1, 0.3, and 0.6% of thiouracil to cockerels from 1-day to 16 weeks of age. Groups of birds were examined at 2-week intervals, starting at 6 weeks of age. The testis weight was depressed on the 0.1% level up to 8 weeks; following this period there was a marked reversal so that by the 14th week the testes of the experimental birds exceeded the normals by about 10 times in weight. Histological examination indicated a slight precocious development of spermatogenesis during the period from 10 to 14 weeks of age. In contrast, the cells of Leydig showed delayed development from the 10th week on, with an increase of intercellular fluid. Depression of the endocrine activity of the cells of Leydig was indicated by reduced rate of comb growth.

Biellier and Turner (12) fed 0.1% thiouracil ration to growing ducks. The testes showed increasing weight in comparison to the controls. The increase was most marked in the 12-week-old group.

Wilwerth *et al.* (131) fed 0.1% thiouracil to Rhode Island Red mature males. They showed a small trend toward lower semen volume and concentration, which was reversed by feeding 0.01 to 0.04% thyroprotein.

E. *Effect of Adrenal Hormones in Normal Female Animals*

Courrier *et al.* (27) reported that the injection of 25 mg. of cortisone acetate per day during various stages of pregnancy in rabbits caused either abortion or intrauterine maceration of the fetuses.

Courrier *et al.* (27) injected 12.5 mg. of cortisone acetate in pregnant rats without interference with normal gestation. Seifter *et al.* (97) reported cortisone caused an increase in the rate of fetal mortality.

Sprague *et al.* (102) injected cortisone acetate in 9 women with normal menstrual function. Of these 3 became amenorrheic during treatment.

F. *Effect of Adrenal Hormones in Hypophysectomized Animals*

Hisaw and Velardo (54) reported that cortisone acetate in doses of 1.5, 5.0, and 10 mg. daily for 4 days did not stimulate growth of the ovaries, uterus, or vagina in hypophysectomized immature rats.

G. *Histology of the Adrenal Glands and Reproductive Performance*

One approach to a study of the adrenal glands as related to reproduction has been the observation of possible abnormalities of histology or cytology in adrenal glands of animals showing reproductive problems. In such studies, naturally, the question of cause and effect is involved. However, these studies may in time be productive.

Weber *et al.* (126), in a study of the normal bovine adrenal gland, described the occurrence of extra medullary myelopoiesis in the cortex of the adrenal in one of every three adult glands. Many of the cows studied were infertile. Cupps *et al.* (30) studied the adrenals of cows with various types of infertility. It was noted that the adrenals of these animals showed a narrowing of the fasciculata, a shrinkage of cells of the reticularis, and a high incidence of extra medullary myelopoiesis. However, Greenstein and Foley (42) have compared the incidence of myelopoiesis in the adrenals of cattle with a normal reproductive history and of abnormal cows. Their data indicated that the condition was not related to fertility or to pregnancy.

Garm (39) noted that the adrenal weights of nymphomaniac cattle were significantly heavier than those of normal cattle of the same age group. It was suggested that the primary cause of the condition was due to the insufficient secretion of luteinizing hormone and persistence of ovarian follicles with prolonged production of estrogen. The estrogen in turn stimulated the pituitary to an increased production of ACTH stimulating the observed increase in adrenal weight. It was suggested also that estrogen exerted a direct action on the zona glomerulosa and

increased the secretion of mineralocorticoids. It was postulated that the mineralocorticoids play a role in the relaxation of the pelvic ligaments.

H. Adrenalectomy and Replacement Therapy in Fowls

Herrick and Torstveit (53) described a successful method of adrenalectomy in fowls. With no treatment the birds died in 6 to 15 hours. By replacement therapy with crude adrenal hormones (eschatin) and NaCl feeding birds have been maintained for more than 80 days. The combs of such fowls become greatly reduced in size and the testes also become smaller and show marked degeneration.

I. Effect of Adrenal Hormones on Egg Production

Kudzia and Champion (64) reported that the injection of 1 mg./lb. body weight of cortisone acetate to laying fowls did not produce a significant reduction in egg production, whereas the 3 mg./lb. level did. Egg weight was not influenced at the lower level of injection.

During cortisone injection at 1 mg./lb. body weight, the fertility of male birds was significantly decreased.

J. The Adrenogenital Syndrome

In the human, adrenal cortical hyperfunction sometimes results in increased secretion of androgen and estrogen. In many such cases tumors of the adrenal are present. In women, the excessive secretion of androgens produces a virilizing condition, shown by excessive hair growth of the male type, including growth of the beard and hair growth on the extremities and chest. The voice becomes masculine. The breasts become reduced in size and the nipples and areola are of the male type. The clitoris is enlarged to the size of a well-developed phallus. The external and internal female genitalia are atrophic. Menstruation ceases completely. These women are strong and athletic.

In men, the excessive secretion of estrogen in this condition has the opposite effect of producing a feminizing syndrome. The breasts enlarge and may secrete milk. Hair growth declines. Atrophy of the testes and penis occurs with loss of libido (135).

The extent of the adrenogenital syndrome in domestic animals is not known. The only obvious symptoms would be reproductive failure and masculinization. To study the extent to which this syndrome causes reproductive failure will require study of the androgen and estrogen excretion in urine and feces, the excretion of 17-ketosteroids and 17-hydroxycorticoids.

P. Hypothyroidism in Birds (Females)

Blivaiss (18) found that thyroidectomized female chicks had juvenile reproductive organs for periods up to two years of age.

Biellier and Turner (12) fed a ration containing 0.1% thiouracil to growing ducks up to 12 weeks for periods of three weeks. In each age group thiouracil depressed the ovarian weight in comparison with the control groups.

Chickens, thyroidectomized at or near maturity, persisted in egg production (105, 132) but at only 10 to 30% of normal. Thyroidectomy not only retarded gonadal size and function but it also reduced the size of the comb and wattles (18) which could be restored with thyroxine injections.

Berg and Bearnse (10) reported that the feeding of 0.1% thiouracil to laying hens caused a significant decrease in rate of lay but had no effect on shell thickness or smoothness.

These observations clearly indicate the marked influence of hypothyroidism upon reproductive performance of male and female birds.

III. ROLE OF THE ADRENAL GLANDS AND THEIR HORMONES IN REPRODUCTION

The determination of the role of the adrenal hormones (the glucocorticoids) in reproduction has been complicated by the relative short survival period of adrenalectomized animals. The profound general effects of adrenalectomy are such as to invalidate conclusions concerning the role of these hormones upon the normal reproductive cycle, pregnancy, parturition or subsequent lactation. However, by replacement therapy the effect of graded dosages of the adrenal hormones may be observed.

A. Replacement Therapy in Adrenalectomized Animals

Replacement therapy of adrenal steroids in adrenalectomized rats to restore estrous cycles, conception, and maintenance of pregnancy has been studied by Cupps (29). Cortisone acetate in graded doses of 0.25, 0.5, and 1.25 mg. daily resulted in a reduced number of implantation sites, few young born alive, and loss of maternal body weight. The 2.5 mg. level increased the implantation sites to normal and increased the number of young born alive. Hydrocortisone acetate at 1.25 mg. level was about as effective as the higher level of cortisone acetate. This level of hydrocortisone plus 0.5 mg. of deoxycorticosterone permitted normal implantation and normal number of young born. Deoxy-

corticosterone in amounts of 0.25, 0.5, and 1 mg daily did not permit reproduction

B Effect of Adrenal Extracts on the Ovary

Casida and Hellbaum (26) cite the earlier literature concerning a relationship between the adrenal glands and the gonads. They reported that extracts from equine adrenals produced ovarian stimulation, as indicated by the presence of large follicles or corpora lutea and evidence of ovulation in 25-day-old rats. The extracts of adrenals of pregnant mares were most potent, but nonpregnant mares and geldings also showed biological activity. Caution should be exercised in interpreting the response to adrenal extracts from pregnant mares because residual blood, high in equine gonadotropin, could possibly be responsible for the gonadal response.

C Effect of Cortisone or ACTH on Deciduomata Production

The experimental production of deciduomata in rats with progesterone is indicative of the role of progesterone in preparing the uterus for the reception of the fertilized egg. Any compound which would depress the production of the deciduomata might be expected to interfere with normal implantation. Hisaw and Velardo (54) reported that 15 mg of cortisone acetate reduced the diameter of deciduomata induced by 15 mg of progesterone in castrate pseudopregnant rats 25%, and 45 mg completely inhibited decidual development. Further, it was shown that the response was reduced 25% by 0.7 mg ACTH daily with complete inhibition following 15 mg.

D Effect of Adrenal Hormones in Normal Male Animals

Ingle (58) reported in earlier studies that 5 to 10 mg daily of cortisone caused some regression of the testes of the adult male rat, whereas in later studies 5 mg daily failed to cause significant loss in the weight of either the testes or seminal vesicles of the adult male rat. Winter *et al* (134) injected 3 mg daily of cortisone acetate for 6 weeks to young adult male rats without effect upon testes weight.

In mature mice the daily injection of 25 mg cortisone for periods up to 17 days resulted in testes, seminal vesicles, and prostate smaller than in the controls (2).

Sprague *et al* (102) reported that continued injection of cortisone acetate in 5 men caused a significant decrease in sex drive and potency.

K. *Pseudohermaphrodisism and Macrogenitosomia Precox*

In children, hereditary adrenal hyperplasia causes pseudohermaphrodisism in females and macrogenitosomia precox in males. This condition is characterized by accelerated growth, muscular development, and epiphyseal ossification. Pubic and axillary hair develops prematurely and other signs of virilization occur. In some cases this condition is already present at birth. The condition results from the excessive secretion of adrenal androgen.

L. *Neutral 17-Ketosteroids in Urine*

These compounds are derived from the metabolism of adrenal and certain gonadal hormones. In normal young, the excretion of these compounds is very slight. As sexual maturity is reached, the excretion of these metabolites increases. Both men and women excrete 17-ketosteroids, but the normal excretion of men is higher than that of women (68).

In cases of the adrenogenital syndrome or pseudohermaphrodisism, the excretion of 17-ketosteroids may increase 2- to 4-fold, indicating the marked increase in the secretion of adrenal androgens. When a feminizing effect is observed in men, increased urinary excretion of estrogens is observed (135).

Study of the 17-ketosteroids in cows' urine has been beset by difficulties due to the presence of certain chromogens which are believed to be carotenoid derivatives. These chromogens parallel the 17-ketosteroids in many respects and thus confound the determination of the latter (75). The reader is referred to the excellent review of the urinary excretion of "reducing corticoids" in cattle and sheep by Holcombe (56). A study of plasma levels of 17-hydroxycorticosteroids associated with acute stress has been presented (93).

M. *Basic Cause of Adrenogenital Syndrome*

The secretion of the hormones of the adrenal cortex is under the control of pituitary adrenocorticotrophic hormone (ACTH). Normally there is equilibrium between the secretion of ACTH and the secretion of the adrenal hormones. When exogenous adrenal hormones are given, there is a corresponding decrease in ACTH secretion and a reduction in the hormones secreted by the adrenal cortex.

It has been demonstrated that the administration of cortisone and other related adrenal hormones will suppress the secretion of the sex hormones and reduce the excretion of 17-ketosteroids in the urine. These experiments suggest that the adrenals of persons with this syndrome may secrete insufficient amounts of the glucocorticoids and ex-

cessive amounts of sex hormones (primarily androgens) under the stimulus of ACTH. The insufficiency of glucocorticoids permits excessive secretion of ACTH. Upon the administration of a glucocorticoid, ACTH secretion is reduced and androgen production decreased.

N. Secretion Rates of the Adrenal Hormones

Before the true role of the adrenal hormones in reproductive processes can be determined, it will be necessary to observe the variation in the secretion rates of these hormones in individual animals and the important factors that influence secretion rate. Progress is being made in this area but so far the methods are chiefly indirect measurements. These studies include perfusion of the isolated adrenal, estimations of the plasma levels, urinary and fecal excretion of adrenal hormones, etc.

With improved estimates of secretion rate, it will then be possible to administer amounts of these hormones within the physiological range. Reproductive performance of animals with hypo- and hyperadrenocorticalism can then be studied. The principles suggested for study of hypo- and hyperthyroidism apply equally well to the adrenal hormones.

O. Interrelation of the Thyroid and Adrenal Glands

There have been many suggestions that a functional relationship exists between the thyroid and the adrenal glands. As improved methods of estimating the secretion rates of the hormones of these two endocrine glands are perfected, it will be possible to determine this relationship more accurately. For the reader interested in this problem, see the review of Money (76) and papers of Robertson *et al.* (92, 93).

IV. ROLE OF OXYTOCIN IN REPRODUCTION

While Dale (31, 32) described the action of posterior pituitary extracts upon the contraction of the uterine musculature fifty years ago, it was not until much later that evidence began to appear indicating that oxytocin might play a true endocrine role in certain phases of the reproductive process concerning uterine motility. Even the extensive use of crude or purified preparations (pituitrin or oxytocin) as an aid in parturition had not proved that oxytocin is normally released at this time. Reports indicating normal parturition in experimental animals hypophysectomized after the middle of pregnancy cast doubt as to the necessity of the posterior lobe hormones in parturition.

In recent years there has been accelerated interest in the role of oxytocin in uterine function from two points of view. First, the release of oxytocin may play a role in the transport of seminal fluid from the

cervix through the uterus and oviducts at the time of coitus. Second, it may act as an aid in the act of parturition.

A. Role of Oxytocin in Semen Transport

Hartman and Ball (48) were the first to show the surprising fact that, within a matter of seconds after ejaculation in the rat, semen was found throughout the uteri. The earlier literature concerning this problem in experimental animals has been reviewed by Hartman (47). Blandau and Money (17) and Blandau (16) have further studied this problem in the rat and Braden (22) in the rabbit.

Recently, a series of reports has appeared concerning the rapid transport of spermatozoa through the reproductive tract of domestic animals. Thus, in the cow (121, 122), in the ewe (103), and in the gilt (25, 33), the ascent of the sperm after copulation appears far too rapid to be accounted for by the normal motility of the sperm. These observations led to the suggestion that uterine motility played a role in the rapid transport of sperm. It was observed that nonmotile sperm and other fluids were also rapidly transported through the reproductive tract (see also discussion of Van Demark (117)).

That increased motility of the uterus at the time of insemination might be due to the release of oxytocin was suggested. It was shown by Hays and Van Demark (50) that the rate of travel of sperm was increased by the injection of oxytocin in the isolated, perfused, bovine uterus, and Rowson (94) observed more rapid transport of radiopaque material in the intact cow. Mann *et al.* (69) not only observed rapid transport of sperm, but several constituents of seminal plasma were found in the uterine horns 40 minutes after mating gilts and 50 minutes after mating the mare. Thus, uterine motility provides not only for the rapid transport of sperm, but for the seminal constituents as well.

It has been shown that maximal rhythmic contractions occur at or near the time of estrus (118, 119, 120) and that tetanic contractions could be caused to occur by massage of the vulva, cervix, and natural mating. Injection of oxytocin produced contractions of a pattern similar to that obtained by cervical massage.

Evidence that increased uterine motility at the time of mating may be due to the release of oxytocin is shown by the fact that milk "let-down" follows the act of coitus. Since it has been shown by a vast amount of experimental work that milk "let-down" is due primarily to the release of oxytocin, these observations are of special significance in implicating oxytocin in uterine motility.

Citation of the early literature concerning this problem has been pre-

sented by Hays and Van Demark (51). Tgetgel (106), in the cow, and Hammond (46), in the mare, observed that manual stimulation of the uterus or mating stimulated milk "let-down." Hays and Van Demark (51) reported that stimulation of the vulva and cervix or natural mating stimulated increased intramammary pressure in most cows.

Further evidence of the release of oxytocin by manual stimulation or the act of mating could be demonstrated by study of the oxytocin content of the blood. Hays and Van Demark (50) reported that blood taken after massage of the vulva and cervix caused contraction of the excised, perfused, cow uteri in each of three trials, whereas blood taken before had no effect. Fitzpatrick (36) extracted oxytocin from blood of cows before and after cervix and uterine stimulations. Bio-assay showed increased amounts of oxytocin after stimulation.

While further evidence of the release of oxytocin at the time of mating or of artificial insemination would be highly desirable, the observations presented are very suggestive. If it is true that sperm transport is facilitated by the release of oxytocic hormone, then two factors should be given increased attention in connection with successful artificial insemination. First, the method of artificial insemination should be such as to provide stimulation of the vagina and cervix comparable to that of natural mating so as to ensure the release of oxytocin. Evidence is available that the amount of oxytocin released by the stimulation of milking varies greatly in animals. It is possible that failure to conceive, in some animals, might be due to failure of sperm transport due to insufficient release of oxytocin. It would be interesting to determine whether the addition of oxytocin to the semen diluters in amounts sufficient to provide adequate uterine stimulation would improve the conception rate.

Second, the inhibiting effect of adrenaline upon the "let-down" of milk is a well-understood phenomenon. It is the basis for the desire on the part of dairymen to maintain "contentment" in the animals at milking time. Excitement of cows leads to adrenaline release which, in turn, blocks the physiological action of oxytocin upon the myoepithelial cells of the mammary gland.

A similar relation between adrenaline and oxytocin appears to be true in regard to uterine motility. Hays and Van Demark (50) observed adrenaline given before oxytocin partially or completely inhibited the increased uterine motility normally induced by oxytocin. This observation suggests the importance of maintaining conditions at the time of the artificial insemination of cattle free from excitement or conditions which would be conducive to the secretion of adrenaline so as to mini-

mize the possibility of adrenaline-blocking uterine motility and normal sperm transport. The futility of attempting to milk excited cows may apply equally to artificial insemination.

REFERENCES

1. Andrews, F. N., and Schnetzler, E. E., *Poultry Sci.* 25, 124 (1946).
2. Antopol, W., *Proc. Soc. Exptl. Biol. Med.* 73, 262 (1950).
3. Aron, M., and Benoit, J., *Compt. rend. soc. biol.* 116, 218 (1934).
4. Asmundson, V. F., *Poultry Sci.* 10, 157 (1931).
5. Asmundson, V. F., and Pinsky, P., *Poultry Sci.* 14, 99 (1935).
6. Atalla, F., and Reineke, E. P., *Federation Proc.* 10, 6 (1951).
7. Benoit, J., *Compt. rend. soc. biol.* 123, 234 (1936).
8. Benoit, J., *Compt. rend. soc. biol.* 125, 459 (1937).
- 8a. Benoit, J., *Compt. rend. soc. biol.* 125, 401 (1937).
9. Benoit, J., and Aron, M., *Compt. rend. soc. biol.* 116, 221 (1934).
10. Berg, L. R., and Bearse, C. E., *Poultry Sci.* 30, 21 (1951).
11. Berliner, V. R., and Warbritton, V., *Proc. 30th Ann. Meeting Am. Soc. Animal Production* p. 137 (1937).
12. Biellier, H. V., and Turner, C. W., *Poultry Sci.* 29, 248 (1950).
13. Biellier, H. V., and Turner, C. W., *Poultry Sci.* 34, 1158 (1955).
14. Biellier, H. V., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 622* (1957).
15. Biely, J., March, B. E., and Silvestrini, D. A., *Poultry Sci.* 33, 1130 (1954).
16. Blandau, R. J., *Am. J. Anat.* 77, 253 (1945).
17. Blandau, R. J., and Money, W. L., *Anat. Record* 90, 255 (1944).
18. Blivaiss, B. B., *Physiol. Zool.* 20, 67 (1947).
19. Blivaiss, B. B., and Domm, L. V., *Anat. Record* 84, 529 (1942).
20. Bogart, R., and Mayer, D. T., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 402* (1946).
21. Booker, E. E., and Sturkie, P. D., *Poultry Sci.* 29, 240 (1950).
22. Braden, A. W. H., *Australian J. Biol. Sci.* 6, 693 (1953).
23. Brody, S., Funk, E. M., and Kempster, H. L., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 278* (1938).
24. Brody, S., and Frankenbach, R. F., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 349* (1942).
25. Burger, J. F., *Onderstepoort J. Vet. Research Suppl. No. 2* (1952).
26. Casida, L. E., and Hellbaum, A. A., *Endocrinology* 18, 249 (1934).
27. Courier, R., Baclesse, M., and Morois, M., *J. physiol. (Paris)* 45, 327 (1953).
28. Crew, F. A. E., *Proc. Roy. Soc. Edinburgh* 45, 252 (1925).
29. Cupps, P. T., *Endocrinology* 57, 1 (1955).
30. Cupps, P. T., Laben, R. C., and Mead, S. W., *J. Dairy Sci.* 39, 155 (1956).
31. Dale, H. H., *J. Physiol. (London)* 34, 163 (1906).
32. Dale, H. H., *Biochem. J.* 4, 427 (1909).
33. du Mesnil du Buisson, F., and Dauzier, L., *Compt. rend. soc. biol.* 149, 76 (1935).
34. Dutt, R. H., and Simpson, E. C., *J. Animal Sci.* 16, 136 (1957).
35. Engle, E. T., see discussion of reference 87.
36. Fitzpatrick, R. J., in "The Neurohypophysis" (H. Heller, ed.), p. 77. Academic Press, New York, 1957.

- 37 Fredeen, H T, *Poultry Sci* 32, 900 (1953)
- 38 Gabuten, A R, and Shaffner, G S, *Poultry Sci* 33, 47 (1954)
- 39 Garm, O, *Acta Endocrinol* 2, Suppl No 3 (1949)
- 40 Glazener, E W, and Jull, M A, *Poultry Sci* 25, 533 (1946)
- 41 Godfrey, G F, *Poultry Sci* 28, 867 (1949)
- 42 Greenstein, J S, and Foley, R C, *J Animal Sci* 16, 341 (1957)
- 43 Greenwood, A W, and Ghu, J P, *Quart J Exptl Physiol* 29, 111 (1939)
- 44 Gutteridge, H S, and Novikoff, M, *Poultry Sci* 26, 210 (1947)
- 45 Gutteridge, H S, and Pratt, J M, *Poultry Sci* 25, 89 (1946)
- 46 Hammond, J, *Vet Record* 48, 519 (1936)
- 47 Hartman, C G, in "Sex and Internal Secretions" (E Allen, ed), 2nd ed, p 630 Williams & Wilkins Baltimore Maryland, 1939
- 48 Hartman, C G, and Ball, J, *Proc Soc Exptl Biol Med* 28 312 (1930)
- 49 Hays, F A, *Poultry Sci* 27, 84 (1948)
- 50 Hays, R L, and Van Demark, N L, *J Dairy Sci* 35, 499 (1952)
- 51 Hays, R L, and Van Demark, N L, *Endocrinology* 52, 634 (1953)
- 52 Henneman H A, Reineke, E P, and Griffin, S A, *J Animal Sci* 14 419 (1955)
- 53 Herrick, E H, and Torstveit, O *Endocrinology* 22 469 (1938)
- 54 Hisaw, F L, and Velardo, J T, *Endocrinology* 49, 732 (1951)
- 55 Hoffman E, and Wheeler, R S, *Poultry Sci* 27, 609 (1948)
- 56 Holcombe, R B, *Acta Endocrinol* 26 Suppl 34 1 (1957)
- 57 Hutt, F B, and Gowe, R S, *Poultry Sci* 27, 286 (1948)
- 58 Ingle, D J, *J Clin Endocrinol* 10, 1312 (1950)
- 59 Jarp, R G *Poultry Sci* 12 322 (1933)
- 60 Jarp, R G, *Proc World's Poultry Congr Exposition 8th Congr* p 136 (1948) Copenhagen
- 61 Jones R G, *Endocrinology* 54 464 (1954)
- 62 Johnson, F N and Meites, J, *Proc Soc Exptl Biol Med* 75, 155 (1950)
- 63 Jull M A, see reference 40
- 64 Kudzia, J J, and Champion, L R, *Poultry Sci* 32, 476 (1953)
- 65 Kumaran, J D S, and Turner, G W, *Poultry Sci* 28 653 (1949)
- 66 Leech, F B and Bailey, G L, *J Agr Sci* 43, 236 (1953)
- 67 Lillie, R J, Sizemore, J R, Milhgan J L, and Burd, H R, *Poultry Sci* 21, 1037 (1952)
- 68 Mason H L, and Engstrom, W W, *Physiol Revs* 30 321 (1950)
- 69 Mann, T, Polge, C, and Rowson, L E A, *J Endocrinol* 13 133 (1956)
- 70 Maqsood M, *Biol Revs Cambridge Phil Soc* 27, 281 (1952)
- 71 Maqsood M, *Fertility and Sterility* 5 382 (1954)
- 72 Maqsood, M, and Reineke, E P, *Am J Physiol* 162, 24 (1950)
- 73 Martinez Campos G, Master's thesis, Michigan State College, East Lansing, Michigan, 1947
- 74 Meites, J, and Chandrasekhar, B, *Endocrinology* 44, 368 (1949)
- 75 Mivner, J P, Saunders, H L, Jr, and Johnson, J E, *J Dairy Sci* 40, 67 (1957)
- 76 Money, W L, *Brookhaven Symposia in Biol No* 7, 137 (1954)
- 77 Oloufa, M M, *Poultry Sci* 32, 391 (1953)
- 78 Oloufa, M M, *Poultry Sci* 33 619 (1954)
- 79 Payne, F, *Anat Record* 88, 337 (1944)
- 80 Petersen, W E, Spielman A., Pomeroy B S, and Boyd W L., *Proc Soc Exptl Biol Med* 46, 16 (1941)

81. Pipes, G. W., and Turner, C. W., *J. Dairy Sci.* 39, 1749 (1956).
82. Pipes, G. W., Premachandra, B. N., and Turner, C. W., *J. Dairy Sci.* 40, 340 (1957).
83. Pipes, G. W., Premachandra, B. N., and Turner, C. W., *Poultry Sci.* 37, 30 (1958).
84. Premachandra, B. N., Pipes, G. W., and Turner, C. W., *J. Animal Sci.* 16, 1063 (1957).
85. Premachandra, B. N., Pipes, C. W., and Turner, C. W., *J. Dairy Sci.* (1958), in press.
- 85a. Premachandra, B. N., Pipes, C. W., and Turner, C. W., *Poultry Sci.* 37, 399 (1958).
86. Reece, R. P., *J. Dairy Sci.* 33, 387 (1950).
87. Reineke, E. P., in "Problem of Fertility" (E. T. Engle, ed.), p. 233. Princeton Univ. Press, Princeton, New Jersey, 1940.
88. Reineke, E. P., and Henneman, H. A., *Proc. Intern. Conf. Peaceful Uses Atomic Energy Geneva* 12, 306 (1956).
89. Reineke, E. P., and Soliman, F. A., *Iowa State Coll. J. Sci.* 28, 67 (1953).
90. Reineke, E. P., and Turner, C. W., *Endocrinology* 29, 667 (1941).
91. Reineke, E. P., and Turner, C. W., *Poultry Sci.* 24, 499 (1945).
92. Robertson, W. G., Mixner, J. P., Bailey, W. W., and Lennon, H. D., Jr., *J. Dairy Sci.* 40, 977 (1957).
93. Robertson, W. G., Lennon, H. D., Jr., Bailey, W. W., and Mixner, J. P., *J. Dairy Sci.* 41, 302 (1958).
94. Rowson, L. E., *Brit. Vet. J.* 111, 334 (1955).
95. Savage, J. E., Turner, C. W., Kempster, H. L., and Hogan, A. G., *Poultry Sci.* 31, 22 (1952).
96. Schultze, A. B., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No.* 392 (1945).
97. Seifter, J., Christian, J. J., and Ehrich, W. E., *Federation Proc.* 10, 334 (1951).
98. Shaffner, C. S., *Poultry Sci.* 27, 527 (1948).
99. Shaffner, C. S., and Andrews, F. N., *Poultry Sci.* 27, 91 (1948).
100. Simpson, S., *Quart. J. Exptl. Physiol.* 14, 162 (1924).
101. Spielman, A. A., Petersen, W. E., Fitch, J. B., and Pomeroy, B. S., *J. Dairy Sci.* 28, 329 (1945).
102. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *A.M.A. Arch. Internal Med.* 85, 199 (1950).
103. Starke, N. C., *Onderstepoort J. Vet. Sci. Animal Ind.* 22, 415 (1949).
104. Stob, M., and Andrews, F. N., *Endocrinology* 51, 165 (1952).
105. Taylor, L. W., and Burmester, B. R., *Poultry Sci.* 19, 326 (1940).
106. Tgetgel, B., *Schweiz. Arch. Tierheilk.* 68, 335 (1926).
107. Thomas, J. W., and Moore, L. A., *J. Dairy Sci.* 36, 657 (1953).
108. Titus, H. W., and Burrows, W. H., *Poultry Sci.* 19, 295 (1940).
109. Turner, C. W., *Poultry Sci.* 27, 155 (1948).
110. Turner, C. W., *Poultry Sci.* 27, 146 (1948).
111. Turner, C. W., Irwin, M. R., and Reineke, E. P., *Poultry Sci.* 23, 242 (1944).
112. Turner, C. W., Irwin, M. R., and Reineke, E. P., *Poultry Sci.* 24, 171 (1945).
113. Turner, C. W., Kempster, H. L., and Hall, N. M., *Poultry Sci.* 25, 562 (1946).
114. Turner, C. W., and Kempster, H. L., *Am. J. Physiol.* 149, 383 (1947).
115. Turner, C. W., and Kempster, H. L., *Poultry Sci.* 27, 453 (1948).

- 116 Turner, C W , and Kempster, H L , *Poultry Sci* 28, 826 (1949)
- 117 Van Demark, N L , in "Mammalian Germ Cells" (G E W Wolstenholme, ed), p 159 Little, Brown, Boston, Mass , 1953
- 118 Van Demark, N L , and Hays, R L , *J Animal Sci* 10, 1083 (1951)
- 119 Van Demark, N L , and Hays, R L , *J Dairy Sci* 34 496 (1951)
- 120 Van Demark, N L , and Hays, R L , *Am J Physiol* 170, 518 (1952)
- 121 Van Demark, N L , and Hays, R L , *Fertility and Sterility* 5, 131 (1954)
- 122 Van Demark, N L , and Moeller, A N , *Am J Physiol* 165, 674 (1951)
- 123 Wallach, D P , and Reineke, E P , *Endocrinology* 45, 75 (1949)
- 124 Ward, D H , M S thesis, Michigan State College, 1950
- 125 Warner, E D , and Meyer, R K , *Endocrinology* 45, 33 (1949)
- 126 Weber, A F , McNutt, S H , and Morgan, B B , *J Morphol* 87, 393 (1950)
- 127 Wheeler, R S , Hoffman, E , and Craham C L , *Poultry Sci* 27, 103 (1948)
- 128 Wheeler, R S , and Hoffman, E , *Endocrinology* 43, 430 (1948)
- 129 Wilson, W O , *Poultry Sci* 28, 581 (1949)
- 130 Wilwerth, A M , M S thesis Michigan State College, 1948
- 131 Wilwerth, A M , Martinez Campos, C , and Reineke, E P , *Poultry Sci* 33, 729 (1954)
- 132 Winchester, C F , *Endocrinology* 24, 697 (1939)
- 133 Winchester, C F , *Poultry Sci* 19, 233 (1940)
- 134 Winter, C , Silber, R H , and Stoerk, H G , *Endocrinology* 47, 60 (1950)
- 135 Wolf, E T , Mills, L C , Newton B L , Tuttle, L L D , Hetting R A , Collins, V P , and Gordon, W B , *J Clin Endocrinol Metabolism* 18, 310 (1958)
- 136 Young, W G , and Peterson, R R , *Endocrinology* 51, 344 (1952)
- 137 Young, W G , Rayner, B , Peterson, R R , and Brown, M M , *Endocrinology* 51, 12 (1952)

CHAPTER 6

Role of the Nervous System in Reproductive Processes

WILLIAM F GANONG

	<i>Page</i>
I Introduction	185
II Regulation of Pituitary Gonadotropin Secretion by the Nervous System	186
A The Evidence for Neural Control	186
1 Effect of Environmental Stimuli	191
2 Effect of Brain Pathology and Lesions	192
B The Mechanism by Which the Nervous System Regulates Pituitary Secretion	196
C Control of FSH Secretion	198
D Control of LH Secretion	201
E Control of Prolactin Secretion	202
F Summary The Regulation of Anterior Pituitary Gonadotropin Secretion	203
III Mating Behavior	211
IV The Onset of Puberty	214
V Parturition—Neural Factors	215
VI Lactation—Neural Factors	216
References	216

I INTRODUCTION

It is apparent that the nervous system is involved, with varying degrees of directness, in almost every aspect of the physiology of reproduction. Reflexes integrated at various levels of the nervous system are involved in sperm transport, parturition, and lactation. Copulation itself is made up of a series of reflexes and reaction patterns integrated into a coordinated whole, and sexual behavior is manifestly a subject for psychological and neurophysiological investigation.

In recent years, increasing attention has been focused on another aspect of the role of the nervous system in reproductive physiology—the regulation by the brain of gonadal function through hypothalamic regulation of anterior pituitary gonadotropin secretion. The details of this regulation are incomplete as yet, but it has become apparent that centers in the brain exercise a major and, possibly, a controlling influence on the amount and type of pituitary gonadotropic hormones liberated into the circulation. These hormones then act on the gonads to bring about, in both sexes, the state of readiness in the reproductive organs and the maturation of the germ cells necessary for successful procreation.

It is apparent, of course, that such preparation would be in vain if it were not associated, in both sexes, with appropriate sexual behavior.

This behavior is known to be dependent on an adequate level of circulating gonadal steroids. Thus the gonads are involved in a kind of "feedback" mechanism to the brain. Pituitary gonadotropin secretion is initiated by the brain; the gonadal hormones are secreted in response to stimulation by these tropic hormones; the gonadal secretions then act back on the brain to initiate the behavior necessary for a successful reproductive performance. In the present chapter, attention will be focused mainly on this brain-to-gonad-to-brain cycle, but copulation, parturition, and lactation will also be briefly discussed from the point of view of the neural components involved, and brief consideration will be given also to the as yet poorly defined, but fascinating, problem of the role of the nervous system in controlling the onset of puberty.

II. REGULATION OF PITUITARY GONADOTROPIN SECRETION BY THE NERVOUS SYSTEM

A. *The Evidence for Neural Control*

The idea that the nervous system regulates gonadal secretion via the anterior pituitary arose originally from observations on the reproductive cycles of normal animals, and information obtained from the analysis of cases of brain disease in humans. The former studies established the fact that sexual cycles in animals were correlated to changes in the seasons, an observation which is difficult to explain except in terms of the intermediation of the nervous system between the environment and the endocrine system. The latter observations established the fact that abnormalities of the testes and ovaries could occur as a complication of disease processes attacking the brain but not demonstrably involving the pituitary or the target endocrine organs.

1. *Effect of Environmental Stimuli*

It has been known for many years, of course, that most birds and many mammals mate only in the spring, although some species, particularly ruminating ungulates, engage in reproductive activity in the fall. Generally, the assumption was made that these endocrine changes were brought about by changes in temperature. Experimental analysis of the numerous variables involved, however, has clearly indicated that probably the most important environmental factor controlling reproductive cycles is the amount of incident light to which the animals are subjected. Rowan points out, in an interesting review, that the effect of light on the reproductive cycles of birds has been put to practical use for centuries in Japan and Holland (164). In both countries, pet songbirds were exposed to increasing amounts of illumination in the fall of the year because it was found that this caused them to sing during the winter.

Singing is associated with testicular development in the males and normally occurs in the mating season. Rowan, in 1925, was the first to approach the problem experimentally (163). He exposed juncos to increasing amounts of artificial illumination during the winter. This species, like other birds, normally shows gonadal development and mating behavior in the spring. Rowan's male juncos showed testicular development in the middle of a Canadian winter, when the temperature was well below zero. These observations on the effect of incident light on gonadal development were extended to mammals by Baker and Ranson (7) and Bissonnette (26). The latter investigator studied the ferret. This animal is particularly valuable for studies of this type because both the male and female normally show gonadal regression during the winter; during the spring, testicular growth occurs in the male and the female goes into estrus. When these animals are exposed to increased illumination during the fall and winter months, the females come into heat and the males show testicular interstitial cell stimulation in midwinter (26).

Since the original observations of Rowan, Bissonnette, and Baker and Ranson, this phenomenon of reproductive photoperiodism has been extensively studied. Much of the work has been descriptive in nature, and there are as yet many unanswered questions about the mechanisms involved. The subject has also been a favorite one for reviews. It will be discussed in this chapter in terms of the stimuli, receptors, and pathways generally involved; the interested reader is referred for details about individual species to the various reviews, particularly those of Hammond (106), Marshall (146), Yeates (203), Zuckerman (206), and Hendricks (119).

In general, photoperiodism is a characteristic of birds and mammals native to the temperate zones of the world. With certain exceptions, tropical animals do not show this dependence on light. Marshall points out that this fact correlates with the relative constancy of day length throughout the year in the tropics (146). Moving an animal native to the northern hemisphere to the southern hemisphere or vice versa initiates, sometimes after a period of irregular cycles, an adjustment of the breeding season and the sexual cycle to the appropriate light changes in the new environment.

The wavelength of the light responsible for initiating reproductive activity has been studied in some species. Generally, light in the far red and infrared portion of the spectrum is ineffective, while, at least in the ferret, the rest of the visible light spectrum is effective, with no selective stimulating effect for any given wave length (147). Marshall and Bowden also observed that ultraviolet radiation is particularly effective in the ferret (148). Artificial illumination from ordinary light bulbs

is effective; in most of the studies of domestic animals, the extra hours of light have been supplied in this fashion (203). For those mammals in which it has been studied in detail, the threshold intensity of illumination necessary to induce stimulation is relatively low. Hammond (106) concludes that once a threshold value is reached, the effectiveness of light is roughly proportional to its intensity up to a maximum, above which further increases in intensity have no greater effect.

The duration of the extra illumination necessary to bring various species into estrus has also been studied. If animals are exposed to a single light interval during each 24 hours, the duration of the exposure is roughly proportional to the degree of stimulation, up to a maximum. However, other factors are involved. In the ferret, daily small increments in the time the animals are illuminated is very effective in bringing about the initiation of estrus. On the other hand, if ferrets brought into estrus by gradually increasing daily light exposure to 18 hours are then subjected to gradual light reduction, they become anestrus when light duration is 16 hours per day (28). Light-darkness alteration is another important aspect of the stimulus to gonadal activity. A number of experiments attest to this fact (105, 115, 129), but perhaps the most dramatic are those of Hart (115), who showed that merely exposing ferrets to an additional hour of light in the middle of the night brought on reproductive activity with great rapidity.

It must be admitted that there is relatively little direct proof that the endocrine effects of light are mediated by the hypothalamus and the anterior pituitary, although the circumstantial evidence is considerable. It is true that hypophysectomized ferrets do not respond to artificial illumination (121), a piece of evidence which is hardly conclusive in itself. Donovan and Harris (65) have reported that sectioning the pituitary stalk blocks the response, a conclusion disputed by Zuckerman (207). Recently, Donovan and his co-workers have reported that hypothalamic lesions accelerate the onset of light-induced estrus in the ferret (67), and Herbert and Zuckerman get the same effect with lesions in the thalamus and caudate nucleus (120). The latter workers interpret their results as indicating that this effect of lesions is due to a nonspecific liberation of gonadotropins along with ACTH in response to the "stress" of operation. It would be interesting to know whether small, carefully located brain lesions can be produced which will block photoperiodic stimulation of the gonads specifically.

That the eye is the receptor involved in reproductive photoperiodism has been shown by numerous experiments in mammals. Thus, for instance, the female ferret blinded by section of the optic nerves either remains in anestrus or comes into estrus at irregular intervals, totally

unrelated to the normal pattern (29). Clark and associates studied the effect of lesions in the visual system on the responses to illumination in ferrets (46). They found that while cutting the optic nerve blocked the response, removal of the entire visual area in the occipital cortex, destruction of the superior colliculi or the ablation of all the lateral geniculate body except its ventral nucleus did not interfere with the normal response to illumination. They suggest that impulses originating in the retina may be transmitted to the hypothalamus via the ventral nuclei of the lateral geniculate body and the accessory optic tracts. Other investigators have described fibers leaving the optic chiasm and tract to enter the hypothalamus directly in the dog (86) and man (117), but functional studies of these fibers have not been reported.

It is true that the pathways involved may be quite different in birds. Benoit, in a series of well-known experiments, which were recently the subject of an excellent review (23), found that male ducks will show testicular development, not only with increased environmental light, but when light is shined on the optic nerves after removing the eye. He also produced increased gonadotropin secretion from the pituitary by shining light directly on the base of the brain and the pituitary itself through a quartz rod (24). He has concluded, therefore, that light receptors in the duck, and possibly in other birds, are located in the brain, and has advanced the hypothesis that the receptor element is somewhere in the ependymal lining of the third ventricle. Although such a mechanism may exist in birds, it is difficult to visualize in mammals, because the thicker skulls in these animals could hardly admit sufficient light to these deep regions of the brain. The studies on optic nerve section in the ferret (29, 46) rule against a diencephalic receptor in this animal.

Hammond (106) feels that the eye is the receptor in mammals, but questions the conclusion that the effect of light reaches the hypothalamus and anterior pituitary from the eye by nerve pathways. He points out, for instance, that section of the optic nerve may destroy important parts of the blood supply to the eye and hence the results of optic nerve section per se are not proof of a neural pathway. Direct proof that the nervous connections between the eye and the hypothalamus mediate the response to increased illumination is therefore a problem for future research, but there is no real evidence for any other pathway.

Rowan offered another explanation for the mechanism of photo-periodic stimulation of the gonads (164). He exposed juncos to an intensity of added light not usually sufficient to stimulate growth of the testes, in a cage with a rotating bar which periodically swept the birds off their perches. He found that this procedure produced gonadal activation and concluded that "wakefulness" was the important factor

in the chain of events leading to gonadotropin secretion. He felt that animals exposed to increased illumination were awake for longer periods and that some aspect of the awake state was responsible for stimulating the hypothalamo-pituitary-gonadal axis. Others have suggested that light increases the general level of metabolism in mammals, and that this, in turn, activates the pituitary (106). It might also be noted, in this regard, that contrary to the views of Selye (182), a release of gonadotropins may accompany the release of ACTH from the pituitary of the stressed female rat (155). However, there is very little evidence to support the hypothesis that the gonad stimulating effects of light are secondary to some type of metabolic stimulation, or "stress," and there is some evidence against it. Marshall points out, for instance, that ferrets used by hunters in fowling become anestrus during the winter, in spite of the increased activity involved, but ferrets staying quietly in their cages, under conditions of increased illumination, become estrus (146). Furthermore, in birds, increased activity or forced exercise does not accelerate gonadal activation, but light does, even in birds trussed up so they cannot move (27, 160).

Another aspect of the problem of photoperiodicity to which little attention has been paid is the phenomenon of refractoriness. Certain animal species exposed to light show gonadal activity and then subsequently develop gonadal regression with continuing increased illumination. The nature and implications of this refractoriness have been discussed in detail by Hammond (106). It can be no more than a subject for speculation in the present stage of our knowledge, but any acceptable theory of the mechanism of photoperiodicity must include an explanation of this phenomenon.

Abrams *et al.* (2) claim that the sympathetic supply to the pituitary is involved in the response to light of the female ferret. They based this conclusion on the fact that removal of the superior cervical ganglia inhibited light-induced estrus. Subsequently, Donovan and van der Werff Bosen (66) claimed that this inhibition was not complete, that the sympathectomized ferrets were capable of response, but required more light than their unoperated controls. The latter workers concluded that the sympathetomy produced miosis; consequently, less light per illumination period fell on the retina.

Environmental factors other than light also play a part in gonadal stimulation, although their role is minor relative to that of incident illumination. In the rat, for instance, low temperatures prolong the estrous cycle (138). Similarly, in the 13-lined ground squirrel, testicular development begins during hibernation in the dark of the burrow, and has been

shown to be dependent on low temperature (200). Other examples of the role of temperature and climate are discussed in Chapter 23.

Some of the components of the reproductive process are apparently partly dependent upon visual contact with other animals of the same species. This is particularly true in birds. The female pigeon, for example, will not ovulate until she sees another pigeon, although, as Matthews showed, she may be tricked into ovulating by seeing her reflection in a mirror (149). The presence of other animals may also be important in some species of mammals (146, 157). In most species of birds, ovulation is related to the number of eggs in the nest. The female stops laying after she has laid a certain number of eggs, the number being remarkably constant for each species. If an egg is removed from the nest each time it is laid, the bird will continue to lay new eggs for a prolonged period of time (145).

Other examples of the importance of environmental exteroceptive stimuli in controlling and adjusting reproductive activity are cited in many reviews (106, 145, 146, 203). In the present context, the important point is that the activity of both the testis and the ovary of many species of animals is modified and regulated by exteroceptive stimuli, presumably acting through afferent nerve pathways to the hypothalamus.

2. *Effect of Brain Pathology and Lesions*

Reports of the effects of brain tumors and infections in humans provide another important source of evidence for regulation of pituitary gonadotropin secretion by the brain. Cases of testicular atrophy in the male and amenorrhea in the female associated with hypothalamic disease but without demonstrable involvement of the pituitary have found their way into the clinical literature for many years. Indeed, gonadal atrophy is a common symptom of ventral hypothalamic disease. Bauer recently analyzed 60 autopsied cases of tumors of the hypothalamus and found hypogonadism in 19 (13). Next to precocious puberty, a condition discussed below, this was the most common endocrine or metabolic symptom of hypothalamic tumor.

The occurrence of obesity and hypogonadism in pubescent males was described by Fröhlich in 1904 (see reference 9). There is considerable debate about the etiology of this syndrome, but there is reason to believe it is hypothalamic in origin (9). Similarly, the occurrence of severe gonadal dysfunction has been reported as a complication of tuberous sclerosis and after meningitis and other infections of the basilar portion of the brain (13).

The effects of hypothalamic disease in the human have been duplicated by experimental lesions in the laboratory. Aschner (5), Camus

and Roussy (42), and Bailey and Bremer (6) were among the early investigators who observed gonadal atrophy following lesions of the hypothalamus that did not involve the pituitary directly. Since that time, the effect on gonadal function of stimulating or destroying various parts of the hypothalamus has been extensively studied, and gonadal atrophy has been observed following hypothalamic lesions in rats, cats, dogs, monkeys, sheep, and a variety of other species. Histological changes have been reported in the pituitary following such lesions, but they seem to be related to altered secretion and are not the changes produced by ischemia or direct involvement by the lesion (31, 32). Clearly, then, the hypothalamus is involved in the control of pituitary gonadotropin secretion.

Other parts of the nervous system are also involved in regulation of pituitary gonadotropin secretion. Some of these are involved only in their role as afferent pathways to the hypothalamus. Included in this category are the nerves from the genital region in species like the cat, rabbit, and ferret, which ovulate only after copulation, and in which ovulation can be induced by stimulating the cervix uteri with a glass rod. Moore and Nalbandov's observations (152, 154) that foreign bodies in the uterus will change the length of the estrous cycle in the ewe may be another example of stimuli from the reproductive tract modifying the time of ovulation, although it occurs in a cyclically ovulating species. The mechanism may be similar to that operating in the chicken, since, in these birds, the presence of a yolk or a foreign body in the magnum inhibits the release of ova from the ovary.

There is scattered evidence indicating that parts of the nervous system, in addition to the afferent pathways discussed above, may play a role in the regulation of gonadotropin secretion. Koikegami's observation (135), subsequently confirmed by Shealy and Peele (184) and Bunn and Everett (39), that stimulation of the amygdala will produce ovulation in some species belongs in this category. However, there is as yet no information on the part played by such areas in the normal physiology of reproduction.

B. The Mechanism by Which the Nervous System Regulates Pituitary Secretion

The demonstrated effects of exteroceptive stimuli and, more particularly, hypothalamic lesions on pituitary gonadotropin release raise the question of the mechanism by which neural influences are translated into changes in pituitary secretion. Two possibilities present themselves: (1) the regulation is nervous, via direct nerve fibers from the central nervous system to the anterior pituitary, and (2) the regulation is

humoral, via substances secreted by the brain and carried in the blood stream to the pituitary.

The first possibility depends on demonstration of a direct efferent nerve supply to the anterior pituitary. There are nerve fibers which reach the anterior lobe from the cervical sympathetic ganglia along blood vessels. Parasympathetic fibers also reach the gland by way of the greater superficial petrosal nerve. Whether or not there are other fibers passing directly from the hypothalamus or neurohypophysis to the anterior pituitary via the pituitary stalk has been the subject of considerable debate. Cajal (41) originally described a scanty supply of such fibers, and Ramussen also found nerve fibers entering the anterior lobe, although he felt they were so few in number that a secretomotor function was unlikely (159). Vasquez-Lopez claimed a more plentiful innervation (196). Harris reviews the controversy in detail in his monograph, and concludes that "the available evidence indicates that the pars distalis receives very few, if any, nerve fibers" (112). Friedgood, on the basis of studies in rats, has claimed a role for the cervical sympathetic fibers in transmitting the "neural stimuli which initiate pseudopregnancy" to the anterior pituitary (see reference 87). The studies of Abrams and co-workers (2) mentioned above, also bear on this problem. However, it is now clear that pseudopregnancy results from a wide variety of unrelated and possibly nonspecific stimuli (190, 194). Furthermore, complete sympathectomy does not prevent ovulation in the rabbit or pregnancy in other species (see reference 37).

There is similarly little evidence for and considerable evidence against the parasympathetic supply to the anterior lobe being of significance in the control of hormone secretion. Thus, although Zacharias produced pseudopregnancy by bilateral removal of the Vidian ganglion (205), Hair and Mezen (104) and Vogt (197) observed normal copulation-induced ovulation in the rabbit after cutting the parasympathetic innervation of the pituitary.

Finally, the question of whether or not there are direct fibers from the hypothalamus to the pituitary becomes a bit academic when the results of cutting the pituitary stalk are considered. This rather complex subject is discussed in detail below. There is general agreement, however, that simple section of the stalk, provided it does not infarct the pituitary or interfere with its revascularization by the portal vessels, permits a return of normal estrous cycles in a relatively short period of time. This interval is too short a period for regeneration of nerve fibers, if such regeneration can indeed be expected to occur from the hypothalamus. Even more convincing are the experiments of such investigators as Greep (97), who actually removed the anterior pituitary from the

pituitary fossa and then replaced it in the sella. Many of these animals resumed normal estrous cycles in relatively short periods of time. This type of experiment makes it unlikely that nerve fibers to the adenohypophysis play an important role as far as secretory function is concerned.

Since control by nerve fibers is unlikely, the humoral pathway becomes, by exclusion, the most likely link between the brain and the anterior pituitary. The hypothesis has thus been advanced that the hypothalamus secretes into the blood stream "neurohumoral" agents which pass to the adenohypophysis to regulate its secretion.

The anterior pituitary is in an excellent position to pick up humoral substances from the median eminence of the hypothalamus because of the existence of the hypophyseal-portal vessels. These blood vessels, a constant anatomical feature in all higher vertebrates (95), arise from capillary loops which penetrate the median eminence at the top of the pituitary stalk. From here, the blood is channeled into sinusoidal vessels which again terminate in capillaries throughout the anterior lobe. The hypophyseal-portal system thus provides a direct pathway from the median eminence to the hypophysis without going through the heart. It is not surprising, therefore, that attention has been focused on the portal vessels as a pathway for transmission of humoral substances from the hypothalamus to the anterior pituitary.

The ease with which these vessels may be interrupted by cutting the pituitary stalk has attracted numerous investigators. One would expect that if the portal vessels were the pathway by which humoral agents regulating tropic hormone secretion were transmitted from the hypothalamus to the pituitary, interruption of these vessels would abolish normal pituitary secretion. Unfortunately, however, it has become abundantly clear that the problem of cutting the pituitary stalk is physiologically much more complex than it seems at first glance. A welter of confusing data has appeared in the literature. The early experiments on stalk section are far too numerous to catalogue here, and the interested reader is referred to reviews of Harris (109, 112) and Zuckerman (206, 207).

Harris brought some order out of this chaos by pointing out that the portal vessels have a remarkable tendency to regenerate (110). He observed that after section of the pituitary stalk, if no scar tissue or other barrier was interposed between the pituitary and the hypothalamus, these vessels would regrow in periods as short as 2 weeks. He therefore advanced the idea of placing between the brain and the pituitary a barrier of some foreign material, such as waxed paper, which would stretch from carotid artery to carotid artery, far enough forward and backward to make regeneration unlikely. Harris also insisted, correctly,

that valid conclusions can be drawn from stalk section experiments only if the animal is injected with India ink or some other material to make detailed visualization of the portal vessels possible. The hypothalamus and pituitary must then be removed in one piece, preferably embedded in celloidon, and serially sectioned. Thick sections are best for bringing out details of the portal vessels. Harris has subsequently performed a series of experiments which meets these criteria. He routinely observes, in the rat and the rabbit, a marked diminution in gonadotropin secretion, the picture of the gonads being that of marked atrophy (112).

Unfortunately, it cannot be said that the experiments that meet Harris' criteria are in themselves conclusive proof that the portal vessels are the essential and only channel for transmission of humoral substances from the brain to the anterior pituitary. In a number of species, at least, it is extremely difficult to cut the portal vessels without destroying the pituitary's own direct arterial supply, because this supply comes to the pituitary through arteries running close to or on the stalk. In others there is no separate supply (55, 56). Even in those species in which stalk section can be performed without the direct arterial supply being compromised, the procedure acutely deprives the pituitary of a large part of its blood supply. It must also be stated that we lack objective criteria at present to prove that the pituitary is functioning as it does only because of the lack of a direct neurohumoral connection to the hypothalamus. This being true, it is important to emphasize, not the many cases in which the gonads and other endocrine target organs are markedly atrophic, but rather the occasional case in which they are not. In this latter group, there are few reported cases following stalk section that also meet Harris' criteria for absence of portal regeneration, but in another group of experiments, those in which the anterior pituitary has been transplanted to a site distant from the hypothalamus, the number is appreciable.

An animal's own anterior pituitary or adeno-hypophyseal tissue from closely related animals may be successfully transplanted. Such transplants have been observed to "take" particularly well in the anterior chamber of the eye and under the capsule of the kidney. These transplanted anterior pituitaries obviously have no direct vascular or nervous connection with the hypothalamus. In the initial, generally short-term experiments with such transplants in hypophysectomized donors, marked gonadal atrophy was usually reported (43, 50, 83-85). However, it is of the greatest significance that, as time has passed, more and more investigators have found reduced but still remarkably high levels of end-organ function in animals in which the pituitary has been transplanted. Generally, these observations have been made on animals with

transplants studied for prolonged periods of time. Thus, Fry and Long (88) have observed partial maintenance of testicular interstitial tissue and seminiferous tubules in one half of a series of rats with transplants in the anterior chamber of the eye; one half of this latter group sired normal litters. Goldberg and Knobil have made similar observations, summarized in detail in a recently published paper (91). Greer and his co-workers, as well as others (101, 180, 181), have also observed a remarkably high uptake of radioactive iodine in such animals, and most investigators agree that these animals are capable of secreting ACTH in response to such stresses as surgical trauma, although their adrenals are usually, but not always, atrophic (140).

How, then, is this mass of data to be interpreted? With improved techniques and more experiments available for analysis, it has become clear that chronic interruption of the portal vessels and all direct nervous connections between the hypothalamus and the pituitary leads to a depression in the release of gonadotropins. However, this depression is not complete, while the gonadal atrophy produced by hypothalamic lesions is as severe as that which follows hypophysectomy (89). It seems reasonable to the present author, therefore, that humoral stimulation can be brought to the pituitary by the general circulation after the portal vessels are interrupted. The humoral agent or agents involved are admittedly much diluted in the general circulation; hence, their effects are quantitatively much less than when they reach the pituitary in the normal fashion down the portal vessels.

It therefore seems that the most likely mechanism by which the hypothalamus regulates anterior pituitary gonadotropin secretion is a neurohumoral one. Certain as yet unisolated humoral substances are secreted in the ventral hypothalamus in response to appropriate stimuli from higher centers. These neurohumors normally pass via the portal circulation directly to the anterior pituitary, where they regulate secretion of gonadotropic as well as other tropic hormones. Cutting the pituitary stalk causes a variable amount of damage to the pituitary, but if the vasculature is chronically interrupted and the pituitary recovers sufficiently, the same humoral agents may be secreted into the general circulation and return to the pituitary, causing a subnormal but still appreciable rate of anterior pituitary secretion.

C. Control of FSH Secretion

The commonest experimental finding relating the hypothalamus to gonadal function is gonadal atrophy in animals with hypothalamic lesions. This atrophy has been reported in both sexes and is usually diffuse, involving all parts of the gonads (25, 31, 32, 61, 109, 137, 173).

However, there is considerable evidence today indicating that the centers concerned with FSH and LH secretion are not entirely coexistent, and some preliminary separation of the mechanisms involved is possible.

The site of lesions producing diffuse atrophy of the gonads is usually in or near the infundibulum. Dey and his colleagues thus describe the lesions which caused ovarian atrophy in their guinea pigs (35, 61, 62). Ganong *et al.* (89), and Daily and Ganong (54) found that, in the dog, destruction of the posterior end of the median eminence, particularly that portion lying immediately around the infundibulum, routinely led to a diffuse atrophy of the gonads in the male dog. Others who have reported testicular atrophy of a diffuse type usually produced lesions in the same area (31, 139).

Dey and associates (62) first reported data suggesting that the centers controlling LH secretion might be at least partially separate from those controlling FSH secretion. They observed in the guinea pig that lesions located in the median eminence "just caudal to the optic chiasm" were associated with a state of constant estrus in the female guinea pig. This observation will be discussed in more detail in the next section. The important point here is that anteriorly placed lesions tend to inhibit the release of LH, while those located in the infundibular region are associated with a diffuse atrophy of the gonads. Undoubtedly, therefore, FSH secretion is inhibited by the latter lesions. Whether or not LH secretion is also inhibited in the female would be difficult to determine, because, in the absence of FSH, follicular development would not occur, and an index of LH activity would therefore be lacking. In the male, on the other hand, selective inhibition of FSH secretion would very likely have little effect on the testes, because LH would presumably stimulate enough androgen secretion to maintain spermatogenesis (98, 198). Lesions in the infundibular stem of the male dog usually produce a picture of interstitial cell and tubular atrophy; in all likelihood, therefore, both LH and FSH secretion are inhibited (57). On the other hand, in preliminary experiments, Davidson and Ganong (57) have observed, in some male dogs with posterior tuberal lesions, a selective destruction of the seminiferous tubules without apparent abnormality of the interstitial tissue. It will be of considerable interest to see if these studies can be confirmed and extended to include data on gonadotropin secretion in such dogs. In the meantime, it can only be concluded that infundibular lesions cause a diffuse atrophy of the gonads in the female and usually in the male.

It is, of course, true that a high level of circulating blood estrogen will inhibit the release of FSH. Where the estrogen acts in this "feedback" mechanism remains an unsettled point. It may be significant that

the transplanted pituitary in a hypophysectomized animal fails to develop castration cells after removal of the gonads (124), although this fact could also be due to ischemia or other damage to the anterior lobe tissue. Recently, Szentagothai has made the interesting claim that anterior hypothalamic lesions in rats, although not causing ovarian atrophy in themselves, prevent the atrophy that usually follows the administration of large doses of estrogen (192). The exact location of these lesions is not clear, but Greer (99, 100) has noted prolonged anestrus without ovarian atrophy in rats with lesions in the preoptic area.

There are numerous studies of the effect of hypothalamic stimulation on LH secretion, but, by contrast, there are no reports of such experiments on FSH secretion. This is perhaps not surprising, since, without some direct measure of FSH secretion, the only possible way to study this problem has been to look for chronic changes in the ovaries and other tissues with chronic stimulation in an anestrus animal, and no successful studies of this type have as yet been reported.

The available data on the hypothalamic control of FSH secretion may be summarized by stating that lesions in the median eminence around the infundibulum lead generally to a diffuse atrophy of the gonads in the female and, possibly with certain exceptions, in the male. Such lesions presumably inhibit secretion of both LH and FSH. There is some suggestive evidence that a separate center concerned primarily with FSH secretion may exist in the tuberal region, but this problem is as yet unsettled.

D. Control of LH Secretion

The experiments of Dey and his colleagues (61, 62, 75) established the fact that anterior hypothalamic lesions are associated with constant estrus in the guinea pig, rather than gonadal atrophy. Dey's lesions in the guinea pig were located just behind the optic chiasma. Hillarp (122) subsequently produced constant estrus in rats by fairly large lesions below the paraventricular nuclei. He also showed that smaller lesions in the tuber cinereum behind the paraventricular nuclei would produce the same effect, and postulated the existence of a fiber tract running posteriorly from the region just below the paraventricular nucleus to the pituitary. Greer observed constant estrus in rats with lesions in the anterior end of the ventromedial nuclei (99). He also observed that the injection of progesterone in these animals would lead to one day of diestrus before the estrous vaginal smear reappeared. His related observation that, in about half of these animals, one course of progesterone would initiate subsequent normal cycling is unexplained. Van Dyke and his colleagues have recently also reported constant estrus after anterior

hypothalamic lesions in rats, they produced luteinization of the multiple ovarian follicles in these animals by LH injection (195). These workers have, like a number of investigators before them, noted an association between hypothalamic obesity and constant estrus, but neither condition was prerequisite for the other.

The problem of the mechanisms controlling LH release has been studied in the rabbit. Ovulation in this animal occurs only after copulation. The act of coitus in some way causes a release into the bloodstream of pituitary "ovulating hormone" (75, 175), most of the available evidence indicates that this "hormone" is predominantly LH. This release from the rabbit pituitary is clearly related to afferent nervous stimulation. It can be produced by stimulation of the cervix uteri with a glass rod. However, genital stimulation is not specifically necessary to bring on ovulation. Thus, ovulation will occur following coitus after local anesthesia of the vagina and neighboring regions (76). It can also occur after a great variety of sensory receptors have been destroyed. Brooks (36, see, however, 201) showed that neither removal of the olfactory bulbs nor destruction of the vestibular apparatus and cochlea would block copulation-induced ovulation. Blinding is also ineffective. In the cat, another species which ovulates only after coitus, complete sympathectomy does not alter the response (37). It appears probable, therefore, that many stimuli converge on the hypothalamus, and that no one afferent pathway is critical to the activation of the brain centers controlling LH release.

Ovulation can be induced in the rabbit by direct stimulation of the hypothalamus, a fact first reported by Harris (107). Apparently this effect is not due to the stimulus spreading to the anterior pituitary cells, because prolonged stimulation of the anterior pituitary with higher voltages than those used to stimulate the hypothalamus did not produce ovulation, except in a few animals (108, 142). As Harris points out, the fact that direct pituitary stimulation produces LH release only with difficulty is also evidence against the response being mediated by nerve fibers ending in the anterior lobe. This is true because, presumably, if small nerve terminals were reaching anterior lobe cells, they would be activated by stimulating current applied to the pituitary, and LH would be released. Harris therefore advances this fact as another piece of evidence in favor of a humoral rather than a neural link between the hypothalamus and the pituitary (112).

The experiments of Markee, Sawyer, Hollingshead, Everett, and their co-workers have also supplied important information about the mechanisms responsible for the release of ovulating hormone in the rabbit and the rat (142-144). These workers have studied in detail

the action of various drugs in both stimulating and inhibiting ovulation in the rabbit.

The observation that copper acetate injected intravenously will induce ovulation in the rabbit was originally reported by Fevold *et al.* (78). Markee and his co-workers added epinephrine to the list of agents causing LH release, although it required huge doses in the previously atropinized animal to produce the effect (142). Brooks and his co-workers showed that copper acetate was ineffective after section of the pituitary stalk (38). This view was subsequently challenged by Sawyer and Markee (169), and whether or not copper acetate, particularly in large doses, can act directly on the pituitary is unsettled. Clearly, however, the other agents that produce ovulation, particularly metrazol and picrotoxin, act primarily on nervous tissue and there is no known evidence of a direct pituitary-stimulating effect for these drugs. The production of LH release and ovulation by the injection of epinephrine in the rabbit, although admittedly requiring large doses of this material, is of interest because it raises the possibility that this substance might play a role in the normal mechanism.

Markee, Sawyer, and their colleagues have also studied in detail the ability of various drugs to block copulation-induced ovulation in the rabbit. They first showed that ovulation could be prevented by large doses of atropine and its congeners, and also by the adrenergic blocking agents, dibenamine and SKF-501 (167, 170-172). Atropine, they found, had to be given within seconds after the copulatory stimulus to have a blocking effect, whereas dibenamine would block if given up to one minute after copulation. This led to the suggestion that the neural mechanism involved included a cholinergic and an adrenergic component, discharge of the former normally preceding the latter. Initially, evidence indicated that the adrenergic discharge was at the median eminence-pituitary level (167). Subsequently, however, Sawyer and his co-workers showed that morphine and Nembutal would block ovulation induced by injecting epinephrine into the third ventricle. For this and other reasons, it was decided that the action of epinephrine was probably somewhere in the brain (168).

It seems clear, therefore, that in the rabbit ovulation is induced by a reflex release of LH under the control of the brain and particularly the hypothalamus. This conclusion is supported, not only by direct electrical stimulation of the hypothalamus, but by both stimulatory and inhibitory pharmacological experiments. Recent experiments have served to confirm and extend these conclusions. Thus, Saul and Sawyer have shown that the ovulation induced by hypothalamic stimulation can be blocked by atropine, Nembutal, and morphine unless the stimulus is

applied directly to the median eminence—in which case ovulation is produced, despite drug blockade (166). Thus, the final common pathway to the pituitary presumably passes through the median eminence.

Everett, Sawyer, and their colleagues have also investigated the role of neural factors in a spontaneously ovulating species, the rat. Working with strains of animals which had an estrous period of remarkably regular length, Everett *et al.* (72) found that they could predict that their rats would ovulate at 4:00 A.M. on the morning following the day of proestrus. Dibenamine or atropine administered between 2:00 and 4:00 P.M. of the day of proestrus would postpone ovulation for one day, and if the drugs were administered repeatedly, ovulation could be delayed for many days. If the drugs were administered before 2:00 or after 4:00 P.M., they were without effect. Therefore, some critical event during this 2-hour time interval must have been inhibited. The event presumably lasts 20–35 minutes and precedes LH release by $\frac{1}{2}$ hour (71, 74). Critchlow (51) reports increased electrical activity of the hypothalamus at this time. Subsequently, it was shown that Nembutal given during this critical period on the day of proestrus would also inhibit subsequent ovulation even though the dose of Nembutal was sufficient to produce no more than mild ataxia (73). These studies have been the subject of an excellent review by Everett (69). Morphine, chlorpromazine, and reserpine were also effective blocking agents when given in this fashion (10–12). Finally, ovulation can be induced in the Nembutal-blocked rat by electrical stimulation of the median eminence (50). Thus, even in spontaneously ovulating species, LH release depends on the activity of the brain. It appears clear, therefore, that the release of LH in the rabbit and the rat, and presumably in other mammals as well, is controlled by a neuroendocrine mechanism integrated in the hypothalamus.

E. Control of Prolactin Secretion

The factors controlling prolactin secretion have been less intensively investigated than those controlling the secretion of the other gonadotropins. Hypothalamic lesions do not seem to alter its output (68), and there is other evidence that the secretion of prolactin is independent of the hypothalamus. It has even been suggested that the hypothalamus functions to inhibit the secretion of this substance (70). It has been observed that following section of the pituitary stalk for advanced mammary carcinoma in humans, the breasts may enlarge and actual lactation take place (103). This occurs in the absence of pregnancy. Its occurrence is difficult to explain, but might be related to an increased secretion of prolactin.

In rats, Everett transplanted the pituitary under the capsule of the

kidney. He found that animals with such transplants had rather severe atrophy of the gonads. However, one week of estrogen therapy in these rats was followed by evidence of prolonged progesterone secretion (70). He suggested from these observations that the transplants secreted prolactin in relatively large amounts. It is possible, therefore, that prolactin is independent of hypothalamic control, or that it is indirectly inhibited by the hypothalamus through the control of FSH secretion. A high level of FSH secretion thus leads to a large production of estrogen from the ovarian follicles, and this estrogen then inhibits the release of prolactin. The relationships between estrogens and prolactin secretion are complicated, however (82), and further experiments in this field are necessary.

There is some evidence that oxytocin stimulates the release of prolactin from the pituitary (49). This mechanism has been invoked to explain the well-known fact that suckling is necessary for prolonged maintenance of the mammary gland in a secretory state. Shibusawa and his colleagues (185-187) also feel that oxytocin is a neurohumor responsible for "gonadotropin" release.

F. *Summary: The Regulation of Anterior Pituitary Gonadotropin Secretion*

The rhythmic release of gonadotropins from the anterior pituitary of the adult female animal has been assumed for many years to be due to a reciprocal relationship between the ovary and the pituitary. According to this view, originally formulated in detail by Moore and Price (151) FSH secretion causes follicular development and consequent increased estrogen secretion. The estrogen thus produced acts on the pituitary to inhibit further FSH secretion and stimulate the release of LH. Ovulation occurs, and a corpus luteum is formed. When the corpus luteum regresses, estrogen and progesterone secretion fall to low levels and the pituitary, released from inhibition, secretes FSH to initiate a new cycle.

The situation is different in the male, since the male pituitary apparently secretes FSH and LH in reasonably constant amounts from day to day. Because ovaries transplanted into castrate males show follicle maturation, but no corpus luteum formation and no cyclic activity (94, 158), it has been claimed that there is a fundamental difference between the male and the female pituitary. Pfeiffer (158) presented evidence that this difference was due to an action of the testis on the pituitary before puberty. He transplanted testes into litter-mate female rats shortly after birth. When these animals reached maturity, they remained in constant estrus, even if the grafted testes were removed. On the other hand, ovaries transplanted into adult males which had been

castrated at birth showed cyclic changes. Accordingly, the idea has become prevalent that the presence of the testis induces an irreversible change in the pituitary of the male.

The results of Harris and Jacobsohn (113) force a reconsideration of this hypothesis. These investigators transplanted pituitary tissue to hypophysectomized female rats from their own young. One to four weeks after transplantation of this tissue under the median eminence the female rats began to cycle normally. The results were the same whether the donor fetus was male or female. More important in terms of Pfeiffer's results, pituitary tissue from adult, litter-mate males also supported normal estrous cycles in the recipient hypophysectomized females (112). Accordingly, the difference between the secretory pattern of the pituitary in the adult male and female is not inherent, but must be due to some other aspect of the environment in which the tissue exists.

Whatever the ultimate determinant of the pattern of pituitary secretion may be, it is clear from the evidence discussed in the preceding sections that the hypothalamus is of great importance in the regulation of gonadotropin secretion in both the adult male and the adult female. The secretion of FSH, and probably of LH as well, is inhibited in both sexes by lesions in the infundibular region of the median eminence. LH secretion in the female may also be selectively inhibited by lesions in the ventral anterior hypothalamus. Conversely, stimulation of the hypothalamus electrically, or with certain drugs, leads to LH liberation. LH secretion may be inhibited by drugs which act on the nervous system and have no known direct action on the adeno-hypophysis. This inhibition may be observed, not only in animals which ovulate only after copulation, but in cyclically ovulating species as well.

How the admittedly important feedback mechanisms from the gonads to the pituitary fit into this pattern of neural regulation is as yet unknown. Another pressing problem is the isolation and chemical characterization of the proposed neurohormones. It is clear, however, that the nervous system is intimately involved in the mechanisms controlling pituitary gonadotropin secretion, and thus, in the regulation of the secretory and gametogenic functions of the gonads.

III. MATING BEHAVIOR

Mating behavior may be legitimately divided into two components. It includes, first, activity consequent to the urge to copulate—the interest in or drive to sexual congress. Secondly, it includes the act of copulation itself, which in turn is made up of an integrated collection of reflexes and reaction patterns, including the necessary postural adjustments, the

pelvic thrusts in the male and the lordotic adjustment of the pelvis in the female, erection, ejaculation, and orgasm.

The reflex arcs and the centers in the nervous system controlling the motor patterns of the sexual act have been studied in considerable detail. It is known, for instance, that most of the postural adjustments for coitus are integrated at the spinal level in both the male and the female (9). Thus, after spinal cord transection, stimulation of the genitalia in the male dog leads to erection and pelvic thrusts, while in the female, perineal stimulation produces elevation of the pelvis.

In the human, erection may be initiated by purely psychic stimuli, but the reaction is primarily a reflex one, initiated by genital stimulation and integrated in the sacral segments of the spinal cord. The efferent pathway is parasympathetic. The motor fibers pass to the genitalia in a relatively well-defined bundle, and since these fibers are also involved in ejaculation, the bundle has come to be called, rather appropriately the "nervus erigens." The vascular engorgement is produced by closure of the so-called "small sluice channels" within the corpora cavernosa, according to some investigators (136), but the main factor involved is arterial dilatation with consequent compression of venous drainage (118).

Ejaculation in the male is initiated by stimulation of the glans, the adequate stimulus being gentle friction and the efferent pathway, the internal pudendal nerve. Semans and Longworthy (183) divide ejaculation into two parts, emission and ejaculation proper. The first event, emission, is the delivery of the semen into the urethra. It is primarily a sympathetic response, integrated in the upper lumbar segments of the spinal cord and carried to the smooth muscle of the vas deferens and associated organs via fibers in the hypogastric plexus. Ejaculation proper follows emission and is the expulsion of the seminal fluid from the urethra. This response is primarily parasympathetic, but it also involves contraction of somatic musculature to aid the expulsion. It is integrated in the upper sacral and lower lumbar portion of the spinal cord, and the motor fibers pass through the internal pudendal nerves and the nervus erigens. Ejaculation can still occur after sympathectomy, according to Kunz (136), but because there is no associated contraction of the internal vesicle sphincter, the ejaculate usually spills into the bladder.

In the female, there is some evidence that, during coitus, uterine contractions occur in response to a spinal reflex, or a neuroendocrine reflex involving the posterior pituitary, or both. Harris (112) discusses in considerable detail the possibility that genital stimulation during coitus initiates a reflex release of oxytocin from the posterior pituitary. According to this theory, the oxytocin then acts on the uterus to initiate a series of contractions of its musculature. These contractions are held to be an

important factor in transporting sperm from the vagina to the Fallopian tube (80). The subject of neuroendocrine posterior pituitary reflexes involving the female reproductive tract is discussed in more detail below.

The reflexes and reaction patterns discussed above are independent or only indirectly dependent on the presence of sex hormones. They are, of course, part of the total act of procreation, and normally occur in an integrated fashion. It is possible to dissect out the various components only by such techniques as destroying various parts of the nervous system. However, the motor activity and reflexes *per se* have little biological meaning except within the broader framework of sexual behavior. This term, as used here, refers to behavior related to reproduction in general, but also particularly to the occurrence of sexual desire. In the male of most species this desire or "rut" is generally present throughout the year, although it waxes and wanes with the seasons to a varying degree (145). In subprimate females, on the other hand, acceptance of the copulatory advances of the male is limited with amazing strictness to a definite period of the sexual cycle. This period of heat or estrus is the most prominent feature of the sexual cycle and that for which the cycle is named.

One of the striking features of sexual behavior is the dependence of normal mating reactions in both sexes on the presence of testicular or ovarian hormones—*androgens* in the male and *estrogens* in the female. The specificity and extent of this dependence has been the subject of spirited debate in the past, and still evokes considerable discussion among students of the problem. It is true, of course, that primates have become largely emancipated from this dependence, and man is almost totally free of it, at least when castrated in adulthood (15, 157). In some species of carnivores, and particularly in the male members of these species, the effects of castration develop slowly, and parts of the normal copulatory reactions may persist indefinitely (16, 93). Generally speaking, however, with few exceptions, castration of adult subprimate mammals, other vertebrates, and birds eventually leads to a general depression, and often to the disappearance, of normal mating behavior. Furthermore, administration of testosterone to the castrated males and estrogen to the castrated females of these species is associated with a return of sexual interest and copulatory behavior (16).

Although estrogens are effective if given in large doses to almost all species, there is some evidence that progesterone is also important in bringing the female into heat. Dempsey reports, for instance, that although estrogen produces heat irregularly in the guinea pig, mouse, and hamster, a course of estrogen followed by progesterone produces it

consistently, and Hammond feels that progesterone is probably necessary in the ewe and the cow (see reference 59 and discussion following).

It is apparent, of course, that the hormones must be acting on some sort of neural substrate to produce their effects on sexual behavior. One plausible theory is that they act to channel the animal's interests and energies into sexual activities. Grunt (102), has noted that castrate male guinea pigs show nondirected hyperactivity, and that this activity is reduced in direct proportion to the extent that a given dose of testosterone tends to restore copulatory activity. Beach has made somewhat similar observations on rats (17). Since the steroids are acting on a substrate, it is not surprising that factors other than the agents themselves are important in determining the response. Thus, Young (204) has emphasized, in the guinea pig, the importance of genetic factors, stimulation from the presence of other animals, and particularly, some previous experience in sexual activity. He states that the latter factor is also important in the dog, the cat, the chicken, and the chimpanzee. The rat is an exception in that the castrate male needs no previous experience to be capable of excellent copulatory performances after androgen treatment.

A source of considerable controversy has been the fact that the individual sex hormones are not entirely specific in their action on sexual behavior. It is well-known in human endocrinology that the administration of testosterone to a homosexual male will usually only intensify his homosexual drive (15). The counterpart of this observation in animals is the extensively documented fact (4, 16, 132) that, at least in certain circumstances, androgens will bring female castrates into heat and estrogens will intensify male copulatory behavior in males. This is true in most of the domestic species, as well as the common laboratory animals. Opinions on the explanation for this nonspecificity range all the way to that of Kinsey and his colleagues (128), who claim that the very term "sex hormone" is unfortunate, these agents simply being responsible for an increase in general metabolic activity, and, therefore, in sexual activity. The subject has been critically reviewed most recently by Goldstein (93), and by Antliff and Young (4). One factor in the reported nonspecificity appears to be the dose of hormone used. Large doses of the heterologous hormone are usually required to elicit mating activity typical of the given sex, while small amounts of the homologous hormone are required. It has also been pointed out that androgen, for instance, in low doses stimulates the male behavior latent in the female, while larger doses stimulate both types. It is true that adrenal steroids and related compounds will induce heat in estrogen-primed ovariectomized guinea pigs, but none of the compounds tested was more than

one quarter as active as progesterone (40). That these compounds were effective in large doses is hardly surprising, since it is a general property of the steroids that while they have one action to a major degree, they have others to a lesser extent. The ability of the adrenal and ovarian steroids to inhibit ACTH release is an example of this phenomenon (176), as is the comparative mineralocorticoid activity of the various steroids in the body, from aldosterone through hydrocortisone to progesterone (94).

Another factor of importance in determining the action of a particular hormone on mating behavior in the castrate animal may be the age at which the animal was castrated. Green and co-workers have recently completed an extensive study of sexual behavior in the cat (96). As a part of that study, they compared the response of male and female kittens to stilbesterol and testosterone with that of postpubertal castrate males and females. They noted that in the prepubertal animals, both male and female, testosterone led to aggressive, boisterous play, with occasional neck gripping and similar activities that might be considered part of the sexual act. Stilbesterol in both sexes produced tail deviation, treading, and, in some cases, the crouched position typical of the copulating female. By contrast, testosterone, as well as stilbesterol, administered to the ovariectomized adult female brought on heat; both substances induced copulatory activity in the castrate male. They conclude, therefore, that prior to puberty the sex hormones have actions on behavior specific to the type of hormone administered, but that after puberty the response is specific to the sex of the animal. These observations are of considerable interest, but the doses of hormones used by Green and his colleagues were large, and it is obvious from the factors mentioned in the preceding paragraph that generalizations about other species would be unwise in the present state of our knowledge.

The actual physiological mechanism by which the gonadal hormones produce their characteristic behavioral effects is unknown. Beach (16) has suggested four general possibilities: (1) an indirect effect through some action on the organism as a whole; (2) an effect through maintenance of structures, such as the genitalia, necessary for particular response patterns, or (3) maintenance of peripheral receptor mechanisms; and (4) an action on the integrative function of the central nervous system. There is no concrete evidence to support the first possibility. The second and third may play a minor role in particular aspects of behavior (19, 20) but there is ample evidence that sexual desire and copulatory behavior can occur in many species in the absence of the genitalia and after complete denervation of the pelvic viscera (8, 9, 36). Similarly, mating, as well as ovulation, occurs in many species after

removal or destruction of a great variety of sensory receptors (9, 36). The last possibility, an action on the central nervous system, thus remains as the most likely explanation.

There has been considerable study of the parts of the brain involved in mating behavior. The areas responsible for the occurrence of heat in the female cat, guinea pig, and rabbit have been particularly studied. In 1940, Bard reviewed his own and other investigators' research on this problem (9). He pointed out that in the spinal and decerebrate female cat only the reflex patterns of copulation were present. Such a preparation shows no interest in the male. Conversely, removal of the entire cerebral cortical mantle does not markedly depress the female's willingness to accept the male. He thus concluded that there was a center, somewhere between the colliculi and the cerebral cortex, which had to be intact for estrous behavior to occur.

Bard pursued this point further by destroying various parts of the diencephalon. For these studies, he used estrogen-treated castrate female cats. He found that when he destroyed the brain anterior to the mammillary bodies, the cats were still willing to accept the male, but that when the brain destruction included the mammillary bodies, heat was absent. Bard also made large mid-hypothalamic electrolytic lesions, but the effect of these lesions on estrous behavior was variable, similar areas of destruction being associated in some animals with loss of heat and in others with its persistence. Dempsey and Rioch (58, 60) used an approach similar to Bard's to study the guinea pig. They found that decortication, large lesions of the pallidum, and destruction of the septal region did not abolish heat in female animals. Decerebration in front of the mammillary bodies was usually not effective in abolishing heat, whereas decerebration behind the mammillary bodies usually was. These experiments led to the hypothesis that there was a "sexual center" in the posterior hypothalamus or upper midbrain.

These conclusions were subsequently challenged by Brookhart, Dey, Ranson and their collaborators (35, 47, 61). Their experiments on the effect of anterior hypothalamic lesions on gonadotropin secretion in the guinea pig have been discussed in the section on neural control of pituitary secretion. They also observed in certain of their animals that some anterior hypothalamic lesions did not induce ovarian atrophy but were associated with an absence of mating behavior in the female guinea pig. In subsequent studies, they found that the injection of anterior pituitary and ovarian hormones in their animals with lesions did not restore mating behavior (34), and that lesions of the pituitary did not block hormone-induced estrus (64). Sawyer and Robinson (173) have also found that small anterior hypothalamic lesions in the cat block the occurrence of

heat, even after estrogen-PMS treatment. These investigators found an essentially homologous area in the mammillary region in the rabbit. There is also some evidence for such a center in the rat (44).

Recently, Clegg and associates have made similar observations in the ewe (48). They found that lesions in the anterior hypothalamus near the median eminence routinely abolished estrous behavior in ewes. Some of these animals had ovarian atrophy, but others appeared to be releasing pituitary gonadotropins in normal fashion. Apparently, therefore, the female sheep, like the female guinea pig and cat, requires an intact center in the anterior hypothalamus for estrous behavior to occur.

The concept of a specific brain center regulating estrous behavior has been criticized by Herbert and Zuckerman (120). These investigators suggest that the "stress" of the lesion, regardless of its site, is responsible for observed changes in reproductive activity. This is a possibility but it seems somewhat unlikely. In the sheep experiments cited above, the animals with lesions which did not block the response continued to cycle regularly, and sometimes mated the day after operation. Absence of mating behavior occurred only in those with a common area of destruction in the ventral anterior hypothalamus (47, 48).

There are apparently no studies of the effect of electrical stimulation of the diencephalon on sexual behavior. Such studies would be of considerable interest, and would help to settle the question of the specificity of the effect of lesions on estrus. A clinical observation of Beattie's may be germane to this subject (45). He calls attention to the occurrence of open, repeated masturbation for short periods postoperatively in usually circumspect, proper ladies who have been subjected to neurosurgical procedures involving the anterior hypothalamus. He suggests that this may be a stimulatory effect. Finally, Kent and Liberman have produced estrous behavior in estrogen-primed hamsters by injecting small doses of progesterone into the lateral ventricle of the brain (127). Harris obtained conflicting results with injections of stilbesterol into the brain of rabbits (112), but the approach is a fascinating one and deserves further use with modern techniques for localized injection.

It seems fair to summarize research on brain centers related to estrous behavior by stating that the bulk of the evidence to date supports the concept of a hypothalamic center concerned with heat in most of the species studied. Without this center heat does not occur, but when it is present, the entire brain anterior to it can be removed without abolishing the response.

The male animal has been less studied, and the problem is more complicated. Beach, in an often-quoted paper, performed partial decortifications in the rat and found that the degree to which sexual activity

was inhibited was correlated with the amount of cortical tissue removed (14). Surprisingly, the effect had no relation to the particular part of the cortex removed. Recently, Beach and his colleagues have repeated these experiments in cats (21, 22, 93), and have found that in these larger animals this generalization breaks down. Thus, damage to the frontal lobes in the male cat tends to diminish sexual performance, but the defect is mostly due to loss of agility. Extensive removal of other areas is generally not effective, although completely decorticate animals will not mate. Beach's generalization that sexual behavior is dependent on the cortex in the male thus seems still valid (159), but it must be modified to include the work of such investigators as Brookhart and his colleagues. These workers observed diminished mating activity in the male guinea pig following lesions in the ventral anterior hypothalamus (33). Clark mentions decreased sexual activity with hypothalamic lesions in rats (44), and Goldstein cites similar results in experiments by Rogers on the hypothalamus in male rats in Beach's laboratory (93).

Hillarp has reported that small lesions in the preoptic area of the hypothalamus induce a temporary state of hypersexual behavior of a male type in both the male and female rat (123). This effect lasts only for a few hours and may well be a stimulatory effect due to irritation around the lesion, rather than an effect of the lesion per se. Where this observation fits into the over-all pattern of the mechanism regulating sexual behavior cannot be answered at the present, but it is reminiscent of Beattie's observation on masturbation cited above.

Hypersexuality is also a characteristic finding after the destruction of certain portions of the temporal lobes. Klüver and Bucy observed increased sexual activity in monkeys, along with other characteristic symptomatology, after removal of the temporal lobes (133, 134). Subsequently, Schreiner and Kling (179) found that lesions in the area of the amygdaloid nucleus were associated with hypersexual behavior in male cats. This temporal lobe nucleus and adjacent structures project to the hypothalamus (90). In Schreiner and Kling's experiments, animals with lesions in this area copulated with any other cat regardless of its sex or size, and attempted to copulate with animals of other species. Castration abolished this hypersexuality, and testosterone restored it in the castrate lesion animals (178). They claim to have found similar hypersexuality in female animals, but their data are not convincing. Subsequently, these investigators have produced hypersexuality in the lynx, the monkey, and the agouti (177).

Green, Clemente and de Groot have re-examined this phenomenon in the course of a series of experiments on the limbic system in cats (96). In general, they have confirmed Schreiner and Kling. However, they find

that the area of destruction common to animals with hypersexuality is not in the amygdaloid nucleus, but next to it, in the pyriform cortex. They also failed to observe changes in the sexual behavior of the female after lesions in this region. Their experiments confirmed the observation of Schreiner and Kling that castration of the male cat with lesion-induced hypersexuality eventually abolished this activity. These interesting and important observations on the relationship between temporal lobe cortex and sexual behavior in the male cat contrast with the previously mentioned observation that removal of all the cerebral cortex in the male cat abolishes mating activity (18, 21, 22, 93). If the latter results are specific, and not secondary to debility, they suggest that a cortical center with an effect on sexual activity opposite to that of the pyriform cortex must exist. Thus, removal of the cortex near the amygdala leads to hypersexuality, while removal of the entire cortex also removes the hypothetical opposing area, and absence of copulatory activity, rather than hypersexuality, results. Possibly, then, the state of sexual interest in the male at any given time is due to the balance struck between these two centers of opposing function. Since such a balance mechanism is involved in the regulation of appetite and also in the adjustment of body temperature, the idea is not without precedent.

The studies reported to date on localization of the brain areas essential for mating behavior may be summarized by stating that in the female of most of the species studied, a hypothalamic "sexual center" exists which must be intact if heat is to occur. There is some evidence that a similar center is involved in the regulation of sexual appetite in the male but, unlike the female, the male apparently requires an intact cerebral cortex for sexual behavior to occur. Removal of the periamygdaloid pyriform cortex in the male cat, monkey, lynx, and agouti results in hypersexual behavior, but because complete decortication results in hypo- rather than hypersexuality, other cortical areas must be involved and the details of the cortical regulation of sexual activity must await further research.

IV. THE ONSET OF PUBERTY

In addition to the part it plays in the endocrine and behavioral aspects of reproduction, there is evidence that the brain may be responsible for the initiation of puberty. This evidence is based on the occurrence of precocious sexual maturity in children with tumors or infections of the diencephalon. The experiments performed by nature in these patients have not as yet been duplicated in the research laboratory, but their implications are far-reaching.

Sexual precocity is a not uncommon condition in humans. It may occur at any age before normal maturity; cases of regular menstruation

in 2-year-old girls have been reported. The syndrome is well described in a recent monograph by Jolly (126). Growth of pubic hair and other manifestations of sexual maturity may occur in association with a particular type of ovarian tumor (the granulosa cell tumor) or with adrenocortical disease, but true puberty—that is, puberty including spermatogenesis in the male and ovulation in the female—is not present in these cases. On the other hand, a sexual maturity that is normal in every respect except its age of onset may be found in association with brain tumors or infections. In certain other cases, no associated pathological process can be detected.

These latter cases with the “constitutional” or idiopathic form of the syndrome are admittedly diagnosed by exclusion. It has been suggested that they merely represent extremes in the normal scatter of the time of onset of puberty. The patients in whom no cause can be found are more frequently girls than boys, but cases do occur in males, and familial cases, suggesting a genetic etiology, have been reported (126, 165).

The cases associated with tumors or infections follow involvement of the pineal gland or the posterior hypothalamus by the pathological process. The occurrence of precocious puberty in patients with localized posterior hypothalamic tumors has been the subject of a number of reports and reviews, especially the well-known review of Weinberger and Grant (199). This important summary has been supplemented by other reports (153, 193). In Bauer's series (13) of 60 autopsied cases of hypothalamic tumor, precocious puberty was present in 24, and was statistically the most common endocrine symptom.

Another rare condition, Albright's syndrome, is characterized by precocious puberty in the female, a fibrous dysplasia of the bones, and a peculiar skin pigmentation along the course of various nerves. Although its etiology is unsettled, Albright thought it was hypothalamic in origin, and 1 autopsied case showed involvement of the posterior hypothalamus by a congenital anomaly (126).

Classically, pineal tumors have also been associated with precocious puberty (131). Why this complication of pineal tumors is found almost exclusively in males is unknown. Kitay and Altschule (131) have published a monumental survey of the world literature on the pineal, and analyzed such cases in detail. Precocious puberty is associated with lesions that destroy the pineal; Kitay and Altschule feel that the accelerated sexual maturity is due to the loss of some specific pineal endocrine secretion. Certainly, the experimental work on the time of onset of puberty in pinealectomized animals is suggestive, but even Kitay's own results are of borderline statistical significance (130). On the other

hand, pineal tumors usually grow posteriorly into the region of the falk cerebri, and thus very rapidly produce posterior hypothalamic compression. Therefore, their endocrine effects may well be secondary to their effects on the hypothalamus. Kitay rejects this point of view, but his evidence against it is indirect. Furthermore, he has no explanation for the occurrence of precocious puberty in association with discrete tumors and other disease processes strictly localized to the posterior hypothalamus.

It has been clearly established by experiments such as those of Foa (81) that gonads from immature animals transplanted into adults function in the adult manner, although there is some evidence that gonads from immature animals are less sensitive to gonadotropin than gonads of the adult (150). Furthermore, the pituitary in the prepubescent animal has a high content of physiologically active gonadotropins (189, 191, 202), yet urinary gonadotropin secretion is low. Therefore, the lack of puberty appears to be due to a failure of the pituitary to secrete gonadotropins. The previously mentioned experiments of Harris and Jacobsohn (113) indicate that this failure of the pituitary to secrete is not due to some peculiarity of the prepubertal pituitary. These workers were able to induce estrous cycles in hypophysectomized female rats by transplanting the pituitaries from male or female fetuses, and cycling commenced before the transplanted tissue would have been expected to reach maturity.

Swingle and his collaborators (191) studied the effect on sexual development of stimulating the uterine cervix electrically in young rats, because such stimulation causes pseudopregnancy and hence, presumably, gonadotropin liberation in the adult animal. The stimulation did accelerate sexual maturation. Mandel and Zuckerman (141) have observed an acceleration in the time of vaginal opening in rats exposed to cold, and interpret their results as being due to a liberation of gonadotropin caused by "stress." This may be true, but in the case of precocious puberty in humans, it must be a minor factor, since other chronic stresses do not accelerate the onset of a sexual maturity in humans.

Recently, two groups of investigators have reported that hypothalamic lesions in female rats accelerate the date of vaginal opening and first estrus (32a, 68a). There is some difference of opinion on the actual location of the effective lesions in these reports. Gellert and Ganong (89a) have confirmed the fact that hypothalamic lesions accelerate the onset of puberty in female rats. They find that the most effective lesions in this respect are in the posterior hypothalamus, just in front of the mammillary bodies. Lesions in the thalamus and the cerebral cortex

also accelerate the onset of vaginal opening to a slight degree, suggesting a nonspecific "stress" effect, as proposed by Mandel and Zuckerman (141). However, it should be emphasized that this acceleration is slight when compared to the marked acceleration produced by posterior hypothalamic lesions.

Summarizing, it may be stated that a variety of pathological processes involving the pineal gland or the posterior hypothalamus are associated with the onset of precocious puberty in children. This puberty is normal except for its time of onset. In animals, it is known that the gonads can respond to gonadotropin, and that the pituitary has a high gonadotropin content before puberty. Transplantation experiments indicate that when properly stimulated, the pituitary in such animals can secrete. Accordingly, the possibility that the brain somehow regulates the time at which puberty occurs is worthy of further investigation.

V. PARTURITION—NEURAL FACTORS

The mechanism initiating and controlling normal labor remains, to a large extent, a physiological enigma. The subject is discussed in detail in Chapter 15. However, two aspects of this problem are germane to the present discussion, and deserve brief consideration here. They are: (1) the role of the posterior pituitary and, more generally, of the hypothalamus in labor; and (2) the role of the uterine nerve supply and spinal reflex arcs affecting the uterine musculature.

It has been known for many years that the posterior pituitary hormone, oxytocin, will cause uterine contractions; its use in human obstetrics to overcome uterine inertia is standard. That oxytocin may also be physiologically important is shown by the fact that electrical stimulation of the hypothalamus in the region of the supraoptic and paraventricular nuclei produces uterine contractions in estrogen-treated or postpartum animals (77, 116). These contractions are independent of direct uterine innervation and presumably are due to release of posterior pituitary hormones. Ferguson (77) reported evidence that distention of the uterus, cervix, or vagina would initiate such contractions; he showed that cord section abolished the response, thus presumably establishing an afferent nervous limb for the reflex release of oxytocin.

Various attempts to measure increased oxytocin levels in the blood late in pregnancy have produced conflicting results, with probably the consensus of opinion supporting the idea of no significant change (see reference 162). However, there is considerable evidence that the uterus is more sensitive to oxytocin at term than it is at other times (161). It is interesting, in this regard, that hypothalamic lesions in pregnant animals frequently produce prolonged abnormal labor (63, 79). On the other

hand, if oxytocin release were important in the onset of normal parturition, one would expect that removal of the posterior lobe of the pituitary or stalk section would cause abnormal labor. Harris has reviewed the effect of such operations on labor (112), and although some instances of abnormal labor have been observed, most of the studies are negative. Furthermore, normal delivery has been observed in humans with diabetes insipidus (30) and in patients in whom the spinal cord has been completely transected. Clearly, therefore, the role of oxytocin-releasing neuroendocrine reflexes in parturition is unsettled, but the problem deserves further study.

That reflexes integrated in spinal centers may also be important in initiating and maintaining labor is shown by the occurrence of prolonged, abnormal labor in animals in which the lumbar spinal cord has been destroyed (125). The afferent pathway for these reflexes is unknown, but it is an old suggestion that pressure of the fetal head in the human on the pelvic visceral nerves and, particularly, on the so-called "cervical ganglion" (136) of the sympathetic system may be important. On the motor side, the uterus has both sympathetic and parasympathetic nerves. Stimulation of its parasympathetic supply induces uterine contraction. On the other hand, delivery can occur after complete denervation of the uterus (188).

From the above, it is obvious that an integrated picture of the action of the nervous system in controlling labor is impossible at present. However, any theory of the mechanism controlling labor must take into account the experimental evidence showing a possible role for the posterior lobe of the pituitary and the spinal cord.

VI. LACTATION—NEURAL FACTORS

Another aspect of reproductive physiology in which the nervous system plays an important role is lactation. The physiology of lactation is considered in detail in Chapter 16. However, no discussion of the role of the nervous system in reproduction would be complete without mentioning the neuroendocrine reflex controlling "milk-letdown"—the ejection of milk from the alveoli and mammary ducts. This subject has been reviewed a number of times, most recently by Cowie and Folley (49).

In recent years, it has become apparent that oxytocin is necessary for milk to be released from the breast (49, 112). Apparently, this hormone causes contraction of the myofibrillary elements in the alveoli and duct walls, with resultant squeezing of the milk out of the nipple. The stimulus that initiates oxytocin release at the appropriate time is suckling, and the afferent pathway begins in touch receptors in the nipple and

areola. Sensory impulses thus set up are relayed to the paraventricular nuclei of the hypothalamus. The neurons in these nuclei, in turn, send impulses down their axons in the supraoptico-hypophyseal tract to the posterior pituitary and stalk region, effecting the release of oxytocin into the blood stream (53). The oxytocin acts on the breast, and milk squirts into the waiting mouths of the young.

There is probably some concurrent release of vasopressin during suckling (52), but evidence has begun to accumulate that the paraventricular nucleus is primarily concerned with oxytocin secretion, and the supraoptic with the release of vasopressin (1, 3). Recently, Olivecrona has shown that paraventricular lesions in rats lead to selective loss of oxytocin from the posterior pituitary without any marked change in vasopressin content (156).

The neuroendocrine reflex controlling milk secretion is thus one of the best worked out of the many mechanisms involving the integrated action of the nervous and endocrine systems in the regulation and control of reproductive phenomena.

REFERENCES

1. Abrahams, V. C., and Pickford, M., *J. Physiol.* **122**, 56P (1953).
2. Abrams, M. E., Marshall, W. A., and Thomson, A. P. D., *Nature* **174**, 311 (1954).
3. Andersson, B., in "The Neurohypophysis" (H. Heller, ed.), pp. 131-140. Academic Press, New York, 1957.
4. Anthoff, H. R., and Young, W. C., *Endocrinology* **69**, 74 (1956).
5. Aschner, B., *Arch. ges. Physiol., Pflüger's* **146**, 1 (1912).
6. Bailey, P., and Bremer, F., *Arch. Internal. Med.* **28**, 773 (1921).
7. Baker, J. B., and Ranson, R. M., *Proc. Roy. Soc. B* **110**, 313 (1932).
8. Ball, J., *J. Comp. Psychol.* **18**, 419 (1934).
9. Bard, P., *Proc. Assoc. Research Nervous Mental Disease* **20**, 551 (1940).
10. Barraclough, C. A., *Federation Proc.* **14**, 9 (1955).
11. Barraclough, C. A., *Anat. Record* **124**, 255 (1956).
12. Barraclough, C. A., and Sawyer, C. H., *Endocrinology* **57**, 329 (1955).
13. Bauer, H. C., *J. Clin. Endocrinol. and Metabolism* **14**, 13 (1954).
14. Beach, F. A., *J. Comp. Psychol.* **29**, 193 (1940).
15. Beach, F. A., *Recent Progr. in Hormone Research* **1**, 27 (1947).
16. Beach, F. A., "Hormones and Behavior." Hoeber, New York, 1948.
17. Beach, F. A., in "Handbook of Experimental Psychology" (S. S. Stevens, ed.), pp. 387-434. Wiley, New York, 1951.
18. Beach, F. A., *Ciba Colloq. Endocrinol.* **3**, 3 (1952).
19. Beach, F. A., and Holz, A. M., *J. Exptl. Zool.* **101**, 91 (1940).
20. Beach, F. A., and Levinson, C. E., *J. Exptl. Zool.* **114**, 159 (1950).
21. Beach, F. A., Zitrin, A., and Jaynes, J., *J. Exptl. Zool.* **130**, 381 (1955).
22. Beach, F. A., Zitrin, A., and Jaynes, J., *J. Comp. and Physiol. Psychol.* **49**, 321 (1958).
23. Benoit, J., and Assenmacher, I., *J. physiol. (Paris)* **47**, 427 (1955).

- 24 Benoit, J, Walter, F X, and Assenmacher, I, *Compte rend soc biol* 144 1206 (1950)
- 25 Biggart, J H, and Alexander, G L, *J Pathol* 48, 405 (1939)
- 26 Bissonnette, T H, *Proc Roy Soc B* 110, 322 (1932)
- 27 Bissonnette, T H, *Quart Rev Biol* 8, 201 (1933)
- 28 Bissonnette, T H, *J Exptl Biol* 12, 315 (1935)
- 29 Bissonnette, T H, *Proc Assoc Research Nervous Mental Disease* 17, 361 (1936)
- 30 Blotner, H, and Kuntz, P, *New England J Med* 227, 287 (1942)
- 31 Bogdanove, E M, and Halmi, M S, *Endocrinology* 53, 274 (1953)
- 32 Bogdanove, E M, Spiritos, B M, and Halmi, M S, *Endocrinology* 57, 302 (1955)
- 32a Bogdanove, E M, and Schoen, H C, *Abstr of 40th Meeting, Endocrine Soc*, p 39 (1958)
- 33 Brookhart, J M, and Dey, F L, *Am J Physiol* 133, 551 (1941)
- 34 Brookhart, J M, Dey, F L, and Ranson, S W, *Proc Soc Exptl Biol Med* 44, 61 (1940)
- 35 Brookhart, J M, Dey, F L, and Ranson, S W, *Endocrinology* 28, 561 (1941)
- 36 Brooks, C M, *Am J Physiol* 120 544 (1937)
- 37 Brooks, C M, *Proc Assoc Research Nervous Mental Disease* 20, 525 (1940)
- 38 Brooks, C M, Beadenkopf, W G, and Bojar, S, *Endocrinology* 27, 878 (1940)
- 39 Bunn, J P, and Everett, J W, *Proc Soc Exptl Biol Med* 96, 369 (1957)
- 40 Byrns, W W, and Shipley, E G, *Endocrinology* 57, 5 (1955)
- 41 Cajal, S R, *Anal soc españ hist nat* [2] 3, 1 August (1894)
- 42 Camus, J, and Roussy, G, *Endocrinology* 4, 507 (1920)
- 43 Cheng, C, Sayers, G, Goodman, L S, and Swinyard, C A, *Am J Physiol* 159, 426 (1949)
- 44 Clark, G, *Am J Physiol* 137, 746 (1954)
- 45 Clark, W E L, Beattie, J, Riddoch, G, and Dott, N M, "The Hypothalamus" Oliver and Boyd, London, 1938
- 46 Clark, W E L, McKeown, T, and Zuckerman, S, *Proc Roy Soc B* 126, 449 (1949)
- 47 Clegg, M T, and Ganong, W F, unpublished observations
- 48 Clegg, M T, Santolucito, J A, Smith, J D, and Ganong W F, *Endocrinology* 62, 790 (1958)
- 49 Cowie, A T, and Folley, S J, in "The Neurohypophysis" (H Heller, ed), pp 183 202 Academic Press, New York, 1957
- 50 Critchlow, B V, *Anat Record* 127, 235 (1957)
- 51 Critchlow, B V, and Sawyer, C H, *Federation Proc* 14, 32 (1955)
- 52 Cross, B A, *J Physiol* 114, 447 (1951)
- 53 Cross, B A, and Harris, J W, *J Endocrinol* 8, 148 (1952)
- 54 Dalt, W J R, and Ganong, W F, *Endocrinology* 62, 412 (1958)
- 55 Daniel, P M, and Prichard, M M L, *Quart J Physiol* 42, 237 (1957)
- 56 Daniel, P M, and Prichard, M M L, *Quart J Physiol* 42, 248 (1957)
- 57 Davidson, J M, and Ganong, W F, unpublished observation
- 58 Dempsey, E W, *Am J Physiol*, 126, 758 (1939)
- 59 Dempsey, E W, *Ciba Colloq Endocrinol* 3 55 (1952)

60. Dempsey, E. W., and Rioch, D. McK., *J. Neurophysiol.* 2, 9 (1939).
61. Dey, F. L., *Endocrinology* 33, 75 (1943).
62. Dey, F. L., Fisher, C., Berry, C. M., and Ranson, S. W., *Am. J. Physiol.* 129, 39 (1940).
63. Dey, F. L., Fisher, C., and Ranson, S. W., *Am. J. Obstet. and Gynecol.* 42, 459 (1941).
64. Dey, F. L., Leninger, C. R., and Ranson, S. W., *Endocrinology* 30, 323 (1942).
65. Donovan, B. T., and Harris, C. W., *Nature* 174, 503 (1954).
66. Donovan, B. T., and van der Werff ten Bosch, J. J., *J. Physiol.* 132, 123 (1956).
67. Donovan, B. T., and van der Werff ten Bosch, J. J., *J. Physiol.* 132, 57P (1956).
68. Donovan, B. T., and van der Werff ten Bosch, J. J., *J. Physiol.* 137, 410 (1957).
- 68a. Donovan, B. T., and van der Werff ten Bosch, J. J., *Nature* 178, 745 (1956).
69. Everett, J. W., *Ciba Colloq. Endocrinol.* 3, 167 (1952).
70. Everett, J. W., *Endocrinology* 58, 788 (1958).
71. Everett, J. W., *Endocrinology* 59, 580 (1958).
72. Everett, J. W., and Sawyer, C. H., *Endocrinology* 45, 581 (1949).
73. Everett, J. W., and Sawyer, C. H., *Endocrinology* 47, 198 (1950).
74. Everett, J. W., and Sawyer, C. H., *Endocrinology* 52, 83 (1953).
75. Fee, A. R., and Barkes, A. S., *J. Physiol.* 67, 383 (1929).
76. Fee, A. R., and Barkes, A. S., *J. Physiol.* 70, 385 (1930).
77. Ferguson, J. K. W., *Surg. Gynecol. Obstet.* 73, 359 (1949).
78. Fevold, H. L., Hisaw, F. L., and Creep, R. O., *Am. J. Physiol.* 117, 68 (1936).
79. Fisher, C., Magoun, H. W., and Ranson, S. W., *Am. J. Obstet. and Gynecol.* 36, 1 (1938).
80. Fitzpatrick, R. J., in "The Neurohypophysis" (H. Heller, ed.), pp. 203-220. Academic Press, New York, 1957.
81. Foa, C., *Arch. ital. biol.* 34, 43 (1900).
82. Folley, S. J., *Recent Progr. in Hormone Research* 7, 107 (1952).
83. Fortier, C., *Endocrinology* 49, 782 (1951).
84. Fortier, C., *Ciba Colloq. Endocrinol.* 4, 124 (1952).
85. Fortier, C., and Selye, H., *Am. J. Physiol.* 159, 433 (1949).
86. Frey, E., *Bull. schweiz. Akad. med. Wiss.* 7, 115 (1951).
87. Friedgood, H. B., in "Textbook of Endocrinology" (R. H. Williams, ed.), pp. 635-698. Saunders, Philadelphia, Pennsylvania, 1950.
88. Fry, E. C., and Long, C. N. H., *Proc. Intern. Physiol. Congr.*, 20th Congr., Brussels, 1950, p. 307 (1956).
89. Ganong, W. F., Fredrickson, D. S., and Hume, D. M., *Endocrinology* 57, 355 (1955).
- 89a. Cellert, R., and Ganong, W. F., unpublished observations.
90. Gloor, P., in "Hypothalamic-Hypophyseal Interrelationships" (W. S. Fields, R. Guillemin, and G. A. Carton, eds.), pp. 74-113. Thomas, Springfield, Illinois, 1950.
91. Goldberg, R. C., and Knobil, E., *Endocrinology* 61, 742 (1957).
92. Goldfien, A., Morse, W. L., Froesch, E. R., Ganong, W. F., Ranold, A. E., and Thorn, C. W., *Ann. N. Y. Acad. Sci.* 61, 433 (1955).
93. Goldstein, A. C., in "Hormones, Brain Function and Behavior" (H. Hoagland, ed.), pp. 97-126. Academic Press, New York, 1957.
94. Goodman, L., *Anat. Record* 63, 223 (1934).
95. Green, J. D., *Am. J. Anat.* 23, 225 (1932).

- 96 Green, J. D., Clemente, G. D., and de Groot, J., *J Comp Neurol* 108, 505 (1957).
- 97 Greep, R. O., *Proc Soc Exptl Biol Med* 34, 744 (1936).
- 98 Greep, R. O., van Dyke, H. B., and Chow, B. F., *Endocrinology* 30, 635 (1942).
- 99 Greer, M. A., *Endocrinology* 53, 380 (1953).
- 100 Greer, M. A., *Recent Progr in Hormone Research* 15, 67 (1957).
- 101 Greer, M. A., Scow, R. O., and Crobstein, C., *Proc Soc Exptl Biol Med* 82, 28 (1953).
- 102 Grunt, J. A., *Proc Soc Exptl Biol Med* 85, 540 (1954).
- 103 Guillemin, R., personal communication
- 104 Hair, G. W., and Mezen, J. F., *Endocrinology* 25, 1965 (1939).
- 105 Hammond, J., Jr., *Nature* 167, 150 (1951).
- 106 Hammond, J., Jr., *Vitamins and Hormones* 12, 157 Academic Press, New York, 1954.
- 107 Harris, G. W., *Proc Roy Soc B* 122, 374 (1937).
- 108 Harris, G. W., *J Physiol* 107, 418 (1948).
- 109 Harris, G. W., *Physiol Revs* 28, 137 (1948).
- 110 Harris, G. W., *J Physiol* 111, 347 (1950).
- 111 Harris, G. W., *Bull Johns Hopkins Hosp* 97, 358 (1955).
- 112 Harris, G. W., "Neural Control of the Pituitary Gland." Williams & Williams, Baltimore, Maryland, 1955
- 113 Harris, G. W., and Jacobsohn, D., *Proc Roy Soc B* 139, 263 (1952).
- 114 Hart, D. S., *J. Agr Sci* 40, 143 (1950).
- 115 Hart, D. S., *J. Exptl Biol.* 28, 1 (1951).
- 116 Haterus, H. O., and Ferguson, J. K. W., *Am J Physiol* 124, 314 (1938)
- 117 Haymaker, W., and Saunders, J. B. de C., *New Intern Clinics* 2, 27 (1940).
- 118 Henderson, V. E., and Roepke, M. H., *Am J. Physiol* 106, 440 (1933).
- 119 Hendricks, S. B., *Am Scientist* 44, 229 (1956).
- 120 Herbert, J., and Zuckerman, S., *Nature* 180, 547 (1957)
- 121 Hill, M., and Parkes, A. S., *Proc Roy Soc. B* 113, 537 (1933).
- 122 Hillarp, N. A., *Acta Endocrinol* 2, 11 (1949).
- 123 Hillarp, N. A., Olivecrona, H., and Silfverskiold, W., *Experientia* 10, 224 (1954).
- 124 Hohlweg, W., and Junkmann, K., *Klin Wochschr* 11, 321 (1932).
- 125 Houssay, B. A., "Human Physiology," 2d ed., McGraw-Hill, New York, 1955
- 126 Jolly, H., "Sexual Precocity." Thomas, Springfield, Illinois, 1955
- 127 Kent, G. C., Jr., and Liberman, M. J., *Endocrinology* 45, 29 (1949).
- 128 Kinsey, A. C., Pomeroy, W. B., Martin, C. E., and Gebhard, H., "Sexual Behavior in the Human Female" Saunders, Philadelphia, Pennsylvania, 1953
- 129 Kirkpatrick, C. M., and Leopold, A. C., *Science* 116, 280 (1952).
- 130 Kitay, J. I., *Endocrinology* 54, 114 (1954).
- 131 Kitay, J. I., and Altschule, M. D., "The Pineal Gland" Harvard Univ. Press, Cambridge, Massachusetts, 1954
- 132 Klein, M., *Ciba Colloq. Endocrinol* 3, 323 (1952).
- 133 Kluver, H., and Bucy, P. C., *A.M.A. Arch. Neurol. Psychiat.* 42, 970 (1939).
- 134 Kluver, H., and Bucy, P. C., *J. Psychol.* 5, 33 (1938).
- 135 Kotkegami, H., Yamada, T., and Uset, K., *Folia Psychiat. et Neurol Japon* 8, 71 (1954).
- 136 Kuntz, A., "The Autonomic Nervous System." Lea & Febiger, Philadelphia, Pennsylvania, 1953

137. Laquer, G. S., McCann, S. M., Schreiner, L. H., Rosenberg, E., Rioch, D. McK., and Anderson, E., *Endocrinology* **57**, 44 (1955).
138. Lee, M. O., *Am. J. Physiol.* **78**, 240 (1926).
139. McCann, S. M., *Am. J. Physiol.* **175** (1953).
140. McDermott, W. V., Fry, E. G., Brobeck, J. R., and Long, C. N. H., *Yale J. Biol. and Med.* **23**, 52 (1951).
141. Mandl, A. M., and Zuckerman, S., *J. Endocrinol.* **8**, 357 (1952).
142. Markee, J. E., Everett, J. W., and Sawyer, C. H., *Recent Progr. in Hormone Research* **7**, 159 (1951).
143. Markee, J. E., Sawyer, C. H., and Hollinshead, W. H., *Endocrinology* **38**, 354 (1946).
144. Markee, J. E., Sawyer, C. H., and Hollinshead, W. H., *Recent. Progr. in Hormone Research* **2**, 117 (1948).
145. Marshall, F. H. A., *Phil. Trans. Roy. Soc. London, Ser. B* **226**, 423 (1936).
146. Marshall, F. H. A., *Biol. Revs. Cambridge Phil. Soc.* **17**, 68 (1942).
147. Marshall, F. H. A., and Bowden, F. P., *J. Exptl. Biol.* **11**, 409 (1934).
148. Marshall, F. H. A., and Bowden, F. P., *J. Exptl. Biol.* **13**, 383 (1936).
149. Matthews, L. H., *Proc. Roy. Soc. B* **126**, 557 (1939).
150. Moore, C. R., *Am. Naturalist* **78**, 120 (1944).
151. Moore, C. R., and Price, D., *Am. J. Anat.* **50**, 13 (1932).
152. Moore, W. W., and Nalbandov, A. V., *Endocrinology* **53**, 1 (1953).
153. Morley, T. P., *J. Clin. Endocrinol. and Metabolism* **14**, 1 (1954).
154. Nalbandov, A. V., Moore, W. W., and Norton, H. W., *Endocrinology* **56**, 225 (1955).
155. Nowell, N. W., and Chester-Jones, L., *Acta Endocrinol.* **26**, 273 (1957).
156. Olivecrona, H., *Acta Physiol. Scand.* **40**, Suppl. 136 (1957).
157. Parkes, A. S., ed., "Marshall's Physiology of Reproduction," 3rd ed., Vol. 1, Part 1. Longmans, Green, London, 1952.
158. Pfeiffer, C. A., *Am. J. Anat.* **58**, 195 (1936).
159. Rasmussen, A. T., *Endocrinology* **23**, 263 (1938).
160. Riley, G. M., *Wilson Bull.* **52**, 73 (1940).
161. Robson, J. M., *J. Physiol.* **88**, 105 (1934).
162. Robson, J. M., "Recent Advances in Sex and Reproductive Physiology," 3rd ed. Churchill, London, 1947.
163. Rowan, W., *Nature* **115**, 494 (1925).
164. Rowan, W., *Biol. Revs. Cambridge Phil. Soc.* **13**, 374 (1938).
165. Rush, H. P., Bilderback, J. B., Slocum, D., and Rogers, A., *Endocrinology* **21**, 404 (1937).
166. Saul, G. D., and Sawyer, C. H., *Federation Proc.* **26**, 112 (1957).
167. Sawyer, C. H., *Am. J. Physiol.* **180**, 37 (1955).
168. Sawyer, C. H., personal communication.
169. Sawyer, C. H., and Markee, J. E., *Endocrinology* **46**, 177 (1950).
170. Sawyer, C. H., Markee, J. E., and Everett, J. W., *Am. J. Physiol.* **166**, 223 (1951).
171. Sawyer, C. H., Markee, J. E., and Hollinshead, W. H., *Endocrinology* **41**, 395 (1947).
172. Sawyer, C. H., Markee, J. E., and Townsend, B. F., *Endocrinology* **44**, 18 (1949).
173. Sawyer, C. H., and Robinson, B., *J. Clin. Endocrinol. and Metabolism* **16**, 914 (1956).

- 174 Saxton, J A, and Greene, H S N, *Endocrinology* 24, 494 (1939)
- 175 Saxton, J A, and Greene, H S N, *Endocrinology* 30, 395 (1942)
- 176 Sayers, G, and Sayers, M A, *Federation Proc* 6, 200 (1946)
- 177 Schreiner, L, and Kling, A, *Am J Physiol* 184, 486 (1956)
- 178 Schreiner, L, and Kling, A, *AMA Arch Neurol Psychiat* 72, 180 (1954)
- 179 Schreiner, L, and Kling, A, *J Neurophysiol* 16, 642 (1953)
- 180 Schweizer, M, and Long M E, *Endocrinology* 47, 455 (1950)
- 181 Scow, R O, and Greer, M A, *Endocrinology* 56, 590 (1955)
- 182 Selye, H, *Ann Internal Med* 19, 403 (1948)
- 183 Semans, J H, and Langworthy, O R, *J Urol* 40, 836 (1938)
- 184 Sherly, G N, and Peele, T L, *J Neurophysiol* 20, 125 (1957)
- 185 Shibusawa, K, Saito, S, Fukuda, M, Koibuchi, E, Kawai, T, and Yamamoto, T, *Endocrinol Japon* 2, 313 (1956)
- 186 Shibusawa, K, Suto, S, Fukuda, M, Kawai, T, Yamada, H, and Tomizawa, K T, *Endocrinol Japon* 2, 183 (1956)
- 187 Shibusawa, K, Saito, S, Fukuda, M, Kawai, T, and Yoshimura, F, *Endocrinol Japon* 2, 47 (1956)
- 188 Simeone, F A, and Ross, J F, *Am J Physiol* 122, 659 (1938)
- 189 Smith, P E, and Dortzbach, G, *Anat Record* 43, 277 (1929)
- 190 Swingle, W W, Seay, P, Perlmutt, J, Collins, E J, Barlow, G, Jr, and Fedor, E J, *Am J Physiol* 167, 586 (1951)
- 191 Swingle, W W, Seay, P, Perlmutt, J, Collins, E J, Fedor, E J, and Barlow, G, Jr, *Am J Physiol* 167, 599 (1951)
- 192 Szengatho, J, 'Symposium internazionale sul diencefalo' Scotti, Milan, 1956
- 193 Troland, G E, and Brown, G H, *J Neurosurg* 5, 541 (1948)
- 194 van der Lee, S, and Boot, L M, *Excerpta Med Sect III* 10, 551 (1956)
- 195 Van Dyke, D G, Simpson, M E, Lepkovsky, S, Koneff, A A, and Brobeck, J B, *Proc Soc Exptl Biol Med* 95, 1 (1957)
- 196 Vasquez-Lopez, E, *J Endocrinol* 6, 158 (1949)
- 197 Vogt, M, *J Physiol* 100, 410 (1942)
- 198 Walsh, E L, Cuyler, W K, and McGullagh, D R, *Am J Physiol* 107, 508 (1934)
- 199 Weinberger, L M, and Grant, F C, *Arch Internal Med* 67, 762 (1941)
- 200 Wells, L J, and Zalesky, M, *Am J Anat* 66, 429 (1940)
- 201 Whitten, W K, *J Endocrinol* 14, 160 (1956)
- 202 Wolfe, J M, and Cleveland, R, *Anat Record* 51, 213 (1931)
- 203 Yates, N T M, in 'Progress of the Physiology of Farm Animals' (J Hammond, ed), Vol I, pp 303-392 Butterworths London, 1954
- 204 Young, W C, in 'Hormones, Brain Function and Behavior' (H Hogeland, ed), pp 75-98 Academic Press New York 1957
- 205 Zacharias, L R, *Endocrinology* 31, 638 (1932)
- 206 Zuckerman, S, *Ciba Colloq Endocrinol* 4, 213 (1952)
- 207 Zuckerman, S, *Ciba Colloq Endocrinol* 8, 551 (1955)

CHAPTER 7

The Estrous Cycle of the Cow

WILLIAM HANSEL

	<i>Page</i>
I. Introduction ✓	224
II. The Nature of the Cycle ✓	224
A. Puberty	224
B. The Length of the Cycle ✓	225
C. The Length of Estrus ✓	225
D. The Time of Ovulation ✓	226
E. Time of Breeding	227
F. The Phenomenon of Metestrous Bleeding ✓	228
G. The Initiation of Estrous Cycles after Parturition ✓	228
III. Changes in Reproductive and Endocrine Organs during the Cycle ..	229
A. Anatomy of the Reproductive Tract ✓	229
B. Ovarian Changes	231
C. Changes in the Oviduct ✓	234
D. Changes in the Uterus ✓	235
1. Changes in the Endometrium	236
2. Changes in the Myometrium	238
E. Changes in the Cervix ✓	239
F. Changes in the Vagina ✓	239
G. Vaginal and Cervical Secretions ✓	242
H. Uterine and Oviduct Fluids ✓	243
IV. <u>Effects of Various Hormones on the Reproductive Tract</u>	243
✓ A. Estrogens and Progesterone	244
1. Effects on the Uterus	244
2. Effects on the Cervix	246
3. Effects on the Vagina	246
✓ B. Effects of Other Hormones on the Bovine Reproductive Tract	246
✓ V. Changes in Other Endocrine Glands during the Estrous Cycle	247
A. The Anterior Pituitary	247
B. The Adrenals and Thyroid	249
VI. ✓ Methods of Altering the Cycle	250
A. Corpus Luteum Removal	250
B. Progesterone Injections	250
C. Gonadotropin Injections	251
D. Oxytocin Injections	253
VII. Ovarian Hormone Levels in Blood and Excreta during the Estrous Cycle	253
A. Estrogenic Substances	253
B. Progesterone	254
VIII. The Mechanism of Ovulation in the Cow	254
A. Neurohumoral Factors Involved in the Ovulation Mechanism	255
B. The Nature of the Neurohumoral Substances	256
C. Present Status of Knowledge Concerning Ovulation in the Cow ..	259
References	260

I. INTRODUCTION

Numerous factors have combined to stimulate research on the estrous cycle and the factors influencing it in the cow. The economic importance of the cow and the losses incurred as a result of lowered fertility in cattle have been major factors in this development. The application of artificial insemination techniques on a large scale has necessitated the development and widespread dissemination of basic knowledge concerning the processes of reproduction in the cow. Because ovulation occurs after the end of estrus and the status of the ovary can be determined by rectal palpation, the cow is an excellent experimental animal for studies on the mechanism of ovulation. The literature concerning the estrous cycle of the cow is voluminous and no attempt will be made to cite all of the studies.

Our knowledge of the estrous cycle, nevertheless, is limited in at least three major areas. Little is known concerning the exact levels of any of the hormones secreted at specific times during the cycle. The exact mechanism of ovulation is still imperfectly understood, and relatively little information is available on how the oviduct performs its functions.

II. THE NATURE OF THE CYCLE

A. Puberty

Age at first estrus in the bovine is markedly influenced by breed and level of nutrition. In a recent study, Sorensen *et al.* (167) found the first estrus occurring at an average age of 49.1 weeks in Holstein heifers raised on a medium level of feeding. The ration consisted of 93% of the total digestible nutrients recommended by Morrison (131). Heifers raised on a high level of feeding, 129% of the medium level, came into estrus at 37.4 weeks of age. In contrast, first estrus did not occur until an average age of 72 weeks in heifers raised on a low level of feeding, amounting to 61% of the medium level. Hansson (92) reported first estrus at 13.3, 12.5, 10.9, 10.4, and 10.6 months of age in heifers of the Swedish Red and White breed raised on levels of 40, 60, 80, 100, and 120% of a standard level, respectively. Joubert (104) also reported earlier maturity associated with higher feeding levels in heifers of several breeds, and Hawk *et al.* (95) presented evidence indicating that growth-inhibiting factors, such as inbreeding and calf-hood scours increased the age at puberty.

Weight at first estrus is less affected by nutrition than age, but large individual variations exist. The average weight at first estrus of Holstein heifers raised on high, medium, and low planes of nutrition (167) were

596, 597, and 532 lb, respectively. Weights at first estrus among heifers raised on the high level of feeding ranged from 394 to 934 lb. Skeletal measurements at first estrus were less variable. The average height of withers at first estrus for heifers on the three descending levels of feeding was 42.7, 44.3, and 44.6 in. The average body lengths at first estrus were 48.5, 50.0, and 49.0 in. for the high, medium-, and low fed groups, respectively. Heifers grown on feeding regimens similar to the high level should be bred for the first time at the second estrus and heifers on a medium-feeding level should be bred at the second or third estrus (167).

B The Length of the Cycle

Many authors have studied the length of the estrous cycle in cows and heifers. There is general agreement that the modal length for heifers is 20 days and 21 or 22 days for cows (7, 26, 27, 130, 139, 150, 175). The mean cycle lengths reported vary from 20.23 ± 2.33 for heifers and 21.28 ± 3.68 for cows to 32.4 days (139).

The percentage of cycles longer than 24 days is reported to be as low as 13 (150) and as high as 45 (139). Estimates of the percentage of cycles less than 18 days in length range from 2.2 (175) to 6.8 (139).

Olds and Seath (139) reported that the repeatability of cycle length is extremely low ($r = 0.069$). The repeatability of breeding efficiency, estimated from data on the regularity of estrus, was found to be 0.18 and the heritability of breeding efficiency based on the same criterion was only 0.05 (Pou *et al.*, 145).

C The Length of Estrus

Estrus is the interval during which an animal will stand when mounted by another cow or a bull. Other manifestations of estrus, such as the flow of clear mucus from the vulva, swollen lips of the vulva, restlessness, bellowing and attempts to mount other females are too variable to be used alone as criteria for detecting estrus. Some heifers show all of these signs of estrus for 12 or more hours before they will stand when mounted by another animal, other heifers exhibit practically none of them as late as one hour before they will stand when mounted.

Asdell (6), summarizing the available data, concluded that the mean duration of estrus was 13.6 hours with a standard deviation of 3.9 hours but more recent studies indicate that this figure should be revised upward to 18 or 19 hours. Trimberger (172) reported heifers in estrus for an average of 15.3 hours and cows for 17.8 hours. A recent series of experiments (84, 85, 89, 103), in which heifers were checked for the

beginning and end of estrus at 2-hour intervals, showed the average length of estrus in normal heifers to be approximately 18 hours. Marion *et al.* (119) found the average length of estrus in heifers, checked for the beginning of estrus at 12-hour intervals and the end of estrus at 2-hour intervals, to be 21.1 hours. Trimberger and Hansel (174) obtained an average figure of 19.3 hours for mature cows checked for the beginning and end of estrus at 2-hour intervals. Wiltbank *et al.* (195) recently reported an average length of estrus of 21.1 hours in Angus heifers checked at 2-hour intervals. The range in length of estrus varies from about 6 to 30 hours in most studies, with a standard deviation of approximately 4.0 hours.

Branton *et al.* (31) have reported that the average length of estrus is only 12-13 hours in cows in a subtropical climate, and that the average length of the cycle is only about 17 days.

Rottensten and Touchberry (152) find real differences among heifers in the degree of expression of heat; these differences have a heritability of approximately 0.21. However, it was concluded that ratings for the degree of expression of estrus are of little or no value in selecting for an increased conception rate. Melampy *et al.* (127) suggested that, following conditioning with estrogen, sexual receptivity in the cow may be due to progesterone produced during the period of the pre-ovulatory development of the Graafian follicle.

D. The Time of Ovulation

According to Asdell's (6) summary, ovulation in the cow occurs on the average 13 to 15 hours after the end of estrus. Subsequent studies, in which the length of estrus and the time of ovulation were determined by estrous checks and rectal palpations at shorter time intervals, place ovulation 10 to 11 hours after the end of estrus. Trimberger (172) reports ovulation in cows was 10.7 hours and in heifers 10.2 hours after the end of estrus. Marion *et al.* (119) report a figure of 9.9 hours for heifers. In each of a series of experiments conducted to study the mechanism of ovulation in the cow, the average time of ovulation was 11 to 12 hours after the end of estrus (84, 89, 103). Ovulations were determined by rectal palpations at 2-hour intervals in these experiments and laparotomies were performed in many cases as a further check. Aschbacher *et al.* (4) also report a figure of 11.1 hours in cows checked for the end of estrus and ovulation at 4-hour intervals. Trimberger and Hansel (174) report an average ovulation time of 10.4 hours after the end of estrus for normal mature cows. According to Wiltbank *et al.* (195), the average time from the end of estrus to ovulation in Angus

heifers, checked at 2-hour intervals, was 9.2 hours. A standard deviation of about 3 hours was found in most experiments with a range of 2 to 22 hours.

The time of ovulation has been altered by several different methods in the cow, and these will be discussed in detail in connection with the hormonal regulation of estrus and ovulation.

E. Time of Breeding

Since the average length of estrus apparently is 18 to 19 hours and the average time of ovulation 10 to 11 hours after the end of estrus, it might be expected that the conception rate would be suboptimal in cows bred during the first 5 hours of estrus, due to a reduction in the fertilizing capacity of the sperm by the time of ovulation. Trimberger's data (172) indicate that this is so, but Vandeplassehe and Paredis (184) obtained pregnancies in cows inseminated before the beginning of estrus and Aschbacher *et al.* (4) have reported fertile inseminations as early as 34 hours prior to ovulation. The latter authors also obtained pregnancies as a result of insemination as late as 14 hours after ovulation. In Trimberger's studies (172) only 31.7% of 60 cows bred after ovulation conceived. He observed a reduction in fertility in cows bred at either the second or sixth hour prior to ovulation. If this reduction in fertility in cows bred shortly before ovulation is real, it suggests that bovine spermatozoa develop a capacity to fertilize, i.e., become "capacitated" only after spending about 6 hours in the uterus or oviduct. This has been shown to be true for rabbit spermatozoa by Chang (44, 45) and by Austin and Braden (9). Interestingly, bull seminal plasma is more effective than either rabbit or human seminal plasma in damaging the fertilizing capacity of previously "capacitated" rabbit spermatozoa (46).

A reduction in the fertility of cattle bred shortly before ovulation may also be related to a limitation in the number of sperm reaching the upper part of the oviduct by the time of ovulation. Van Demark and Moeller (182) have shown, however, that some sperm reach this area within a few minutes after insemination. The entire question of sperm "capacitation" and transport in the bovine needs further study.

In practice, the time of insemination appears to be of importance in artificial breeding operations. Bearden (13) found a 60-90 day non-return rate of 64.1% for cows not turned out for detecting estrus during the winter months as contrasted to percentages of 69.5 and 70.4 for cows turned out once and twice daily. The nonreturn rate in herds of 10-29 cows was 70.3% while in herds of 60 or more cows it was

65.6%. These differences are probably related to better timing of inseminations resulting from more accurate information regarding estrus in the smaller herds and the herds turned out more frequently. A recent survey by Foote (138) indicates that the current recommendation to inseminate cows in the afternoon if in estrus that morning, or before noon if in heat the preceding afternoon results in adequate fertility under practical conditions. Experience gained in studying a large number of herds with breeding problems indicates that heat periods are often missed because dairymen do not watch their cows closely enough. On the other hand, many cows are bred too soon because the inseminator is called before the cow comes in "standing" estrus.

F. The Phenomenon of Metestrous Bleeding

Metestrous bleeding occurs at 1 to 3 days postestrus in about 90% of the cycles in young heifers and in about 50% of the cycles in mature cows (86, 108, 171). It can occur even before the end of estrus. The lower incidence of metestrous bleeding in mature cows may be related to the fact that the endometrial arterioles are more highly coiled in older cows than in heifers (83). The uterus is the source of the blood and bleeding occurs both by diapedesis and as a result of capillary rupture (86, 186). The occurrence of the bleeding is unrelated to whether or not conception occurs in animals bred during the associated estrous period (171). Two reports (10, 112) show that 20 to 30% of cows conceive when bred at the time of metestrous bleeding.

Ovulation is not a prerequisite to metestrous bleeding, since bleeding sometimes starts before ovulation. Hansel and Asdell (86) found bleeding in heifers ovariectomized during estrus, and at 1 day postestrus. Ovariectomy at other stages of the cycle was not followed by metestrous bleeding. Metestrous bleeding could not be produced in ovariectomized heifers by treatment with any of a variety of hormones and combinations of hormones, even though the declining level of estrogens in the blood after estrus appears to be one of the hormonal factors involved (86).

G. The Initiation of Estrous Cycles after Parturition

Estimates of the average length of the interval from parturition to first estrus in dairy cattle ranged from 32 to 69 days (27, 34, 47, 98, 10, 175). Estimates of the average length of this interval in beef cattle range from 51 to 80 days (77, 185). All workers agree that the length of this interval is quite variable and that endometritis prolongs it. There is also agreement that some cows ovulate one or more times before

showing signs of estrus for the first time after parturition (41, 175). Clapp (50) found the interval between calving and first estrus to be considerably longer in cows milked three and four times a day and in nurse cows than in cows milked twice a day. Casida and Wisnicky (41) reported a long interval (60.4 days) and a high incidence of "quiet" ovulations (68%) in cows milked three times daily. These observations may be of some significance in the light of newer knowledge of the mechanism of ovulation, to be discussed in a later section.

Estimates of the average time necessary for involution of the uterus after calving range from 26 to 47 days (34, 37, 41). Level of production appears to have little influence on the length of time from parturition to first estrus (98, 140). Buch *et al.* (34) found cows calving in the summer months returning to estrus sooner than cows calving in the winter months, but Herman and Edmondson (98) noted no effect of season of calving. Warnick (185) reported that beef cows calving late in the spring returned to estrus sooner than cows calving earlier, and that age of the cow at calving had no effect on the interval between parturition and first estrus. Herman and Edmondson (98), working with dairy cattle, found the longest interval in first calf heifers 1.5 to 2.5 years of age and the shortest interval in cows 2.5 to 7 years of age. The length of the interval increased again in cows over 7 years of age. Buch *et al.* (34), however, reported that the uterus involutes faster in primiparous than in multiparous cows.

According to Olds and Seath (140) the repeatability of first estrus after calving is 0.29, and the heritability 0.27, but Warnick (185) reported a repeatability of only 0.06 in beef cattle. A repeatability of 0.19 for the postpartum interval in dairy cattle was reported by Chapman and Casida (48).

Numerous studies (48, 64, 102, 160, 173, 181) on the fertility of dairy cattle bred at various intervals after calving indicate that a minimum interval of 50 days is necessary for satisfactory fertility, and that it is probably unwise to breed dairy cows sooner than 60 days after calving. Similar results have been found in beef cattle (113, 185).

III. CHANGES IN REPRODUCTIVE AND ENDOCRINE ORGANS DURING THE CYCLE

A. Anatomy of the Reproductive Tract

The anatomical features of the bovine reproductive tract have been described in detail by several authors (8, 151, 164), and will be considered only briefly here. The major parts of the tract are shown in Fig. 1, Volume II, Chapter 5.

The ovaries, in addition to producing ova, produce hormones conditioning the accessory reproductive organs, bringing the cow into estrus, and regulating the course of pregnancy, at least in its initial stages. Usually only one follicle ruptures and one ovum is liberated at the time of ovulation but multiple ovulations sometimes occur. The right ovary sheds the ovum in about 60% of the estrous cycles (51, 144, 147); its average weight is larger than the left ovary, even in calves (36, 147). Apparently, ova are rarely lost into the abdominal cavity in the cow, since fertilized ova have been recovered from the oviduct 3 days after breeding in 97% of normal heifers bred to high fertility bulls (12). As fertilization occurs in the ovarian third of the oviduct, sperm transport is a major function of the oviduct. Some sperm reach the ovarian end of the oviduct within a few minutes after insemination (182) by a mechanism incompletely understood. After fertilization, the oviduct transports the dividing ovum to the uterus, a process usually requiring about 4 days.

The uterus must furnish nutrients to and remove the waste products from the growing fetus. Because implantation does not occur until about the 30th day of pregnancy (128), these functions are complex. Furthermore, the uterus must accommodate the tremendous growth occurring in the bovine fetus prior to parturition. The bovine uterus consists of two cornua which merge into a relatively short body of the uterus, and the cervix uteri. The luminal surfaces of the uterine horns are characterized by caruncles arranged in four rows which, during pregnancy, serve as sites of attachment for the fetal placenta.

The cervix uteri usually consists of four folds of dense connective tissue covered by mucus-secreting epithelial cells. The opening of the cervix into the anterior vagina is referred to as the os uteri. The vagina and vulva comprise the remainder of the reproductive tract. The external urethral orifice opens into the ventral surface of the vulva, and just posterior to this point the suburethral diverticulum, a blind sac, is found. Gartner's ducts open into the vulva just lateral to the urethral orifice. The ducts from the vestibular glands open into the vulva posterior and lateral to Gartner's ducts. The clitoris, the homolog of the penis in the male, is located just anterior to the lips of the vulva.

Perkins *et al.* (144) measured various parts of the reproductive tract of 100 parous cows at unknown stages of the estrous cycle and reported the following mean values: length of vagina (including vulva), 17.2 cm.; length of cervix, 8.0 cm.; length of left uterine horn, 39.0 cm.; length of right uterine horn, 39.6 cm.; length of left oviduct, 20.7 cm.; and length of right oviduct, 20.7 cm. Sorensen *et al.* (167) reported average

lengths of 22.5, 22.6, and 23.3 cm. for the oviducts, uteri, and vaginas of 64-week-old heifers.

The uterus is attached to the abdominal wall by the broad ligament, or mesometrium. It receives its blood supply from the middle uterine arteries. The ovaries receive blood from the utero-ovarian arteries which may anastomose with the middle uterine arteries in the broad ligament. A branch of the internal pudic artery supplies the vagina and the cervical region of the uterus (83).

✓ B. Ovarian Changes

The bovine ovary consists of a cortex of connective tissue stroma, in which the follicles containing the ova are imbedded, and a medulla containing connective tissue and many blood vessels. The dense layer of connective tissue underlying the surface is called the tunica albuginea. The arterioles entering the hilus of the ovary are remarkably coiled (83). The surface layer of flattened cells is called the germinal epithelium. It has been suggested that ovogenesis occurs from this layer in the adult animal, but recent work by Mandl *et al.* (116) indicates that this does not occur, at least in the rat. Brambell (30) has reviewed the problem of whether or not ovogenesis occurs in adult mammals.

Many atretic follicles are found in the ovaries of prepuberal heifers (167). Hoffinger (101) observed typical atretic follicles consisting entirely of connective tissue elements and atypical atretic follicles containing lutein cells in the ovaries of calves during the first month of life. Follicular growth decreased at 3 months of age and then increased until shortly before sexual maturity.

After puberty is reached, the primary follicles, consisting of an ovum surrounded by a single layer of epithelial cells, begin the development which will eventually culminate in ovulation. The follicle grows by a multiplication of this single layer of cells surrounding the ovum and by a special development of the surrounding connective tissue cells, the theca. The theca develops into two layers, interna and externa. The theca interna cells are believed to be the source of estrogenic hormones. The single layer of cells surrounding the ovum proliferates and these granulosa cells secrete the follicular fluid which results in the formation of an antrum in the follicle (Fig. 1). The cells surrounding the ovum, known as the cumulus oophorus, eventually become separated from the granulosa cells lining the cavity of the follicle. The follicular fluid contains some of the estrogens secreted by the theca interna. It has a protein content of 4.65 to 5.60%, and contains 39-43 mg. per 100 ml. of glucose, 12-95 mg. per 100 ml. of lactic acid, and 0.8-3.0 mg. per

100 ml. of ascorbic acid (115). The proteins contained in the follicular fluid are qualitatively, but not quantitatively, similar to those in the blood serum (35).

The follicle grows rapidly during the 3 days prior to estrus. Some average figures for the diameter of the largest follicle found in the ovaries of 48-80-week-old Holstein heifers are as follows: 1-4 days post-estrus, 13 mm.; 5-8 days postestrus, 11 mm.; 9-13 days postestrus, 14 mm.; and 18-21 days postestrus, 20 mm. (91).

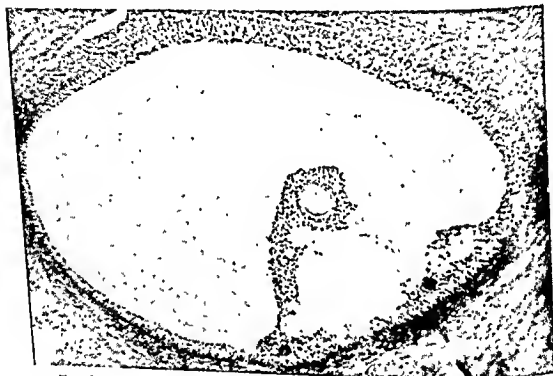


FIG. 1. Cross section of a developing follicle in a bovine ovary showing the ovum, cumulus oophorus, granulosa cells, and theca. Magnification: $\times 94$.

Ovulation is the rupture of the follicle at the surface of the ovary and the release of the ovum with its cumulus oophorus into the infundibulum of the oviduct. The exact mechanism concerned in the rupture of the follicle wall is unknown, but pressure from the follicular fluid is probably only one of the factors involved. Actually, there is a decrease in follicular tension in the bovine which is noticeable on rectal palpation of the ovaries at 2 to 4 hours prior to ovulation.

After ovulation, a small amount of blood escapes into the empty follicle, forming a clot (126). The granulosa cells enlarge and become filled with droplets of a yellow lipid material to form the corpus luteum. Strands of connective tissue from the theca invade the clot and some of

these cells are probably transformed into lutein cells. There is still some question as to whether this transformation occurs (30). There is also some diversity of opinion as to whether the granulosa cells undergo mitotic divisions in the developing corpus luteum (30, 126, 143). The corpus luteum secretes progesterone, which prepares the uterus to receive the fertilized ovum and is essential for the maintenance of pregnancy.

Hansel and Trimberger (87) noted that the nuclei of the granulosa cells become densely chromatic and the cytoplasm vacuolated in heifers in estrus and prior to ovulation, and suggested that these changes might be related to an increased secretion of progesterone prior to ovulation—an idea that receives some support in that progesterone is found in bovine follicular fluid near the time of ovulation (61).

The corpus luteum increases in size until about the 16th to 18th day of the cycle and then decreases in size shortly before the cow returns to estrus. Some average figures for the diameter of the active corpus luteum in the ovaries of Holstein heifers 48–80 weeks of age are as follows: 1–4 days postestrus, 8 mm; 5–9 days postestrus, 15 mm; 9–13 days postestrus, 18 mm; 14–18 days postestrus, 20.5 mm; and 18–21 days postestrus, 12.5 mm. In the event of pregnancy the corpus luteum persists.

Sykes *et al* (169) and Moss *et al* (132) studied alkaline phosphatase activity and glycogen distribution as affected by the estrous cycle. Alkaline phosphatase is present in both the theca and granulosa layer in the primordial follicle. As the follicle develops, the phosphatase in the granulosa decreases and that in the theca increases, so that in medium to large follicles none is seen in the granulosa. In preovulatory follicles, phosphatase reappears in the granulosa, and the concentration in the theca declines. Phosphatase in the ovum is limited to the nucleus. The zona pellucida also contains a heavy concentration of phosphatase. The corpus luteum contains large amounts of phosphatase until the 13th day of the cycle, but little or none is present in later stages of the cycle.

In the developing follicle, glycogen is found in the ovum and in the cumulus oophorus and the granulosa. It is absent in the theca. Luteal cells do not contain glycogen. Ovarian structures which contain phosphatase do not contain glycogen concurrently. A layer of phosphatase rich tissue frequently intervenes between the circulation and structures containing glycogen.

Cysts of various sizes have been noted in bovine corpora lutea by several authors, who considered them either as a normal stage of de-

velopment (79) nor as an abnormal condition of little consequence (7). Recently, however, McEntee (125) has found a surprisingly high incidence (12%) of luteal cysts over 1 cm. in diameter in nonpregnant cattle sent to slaughter. No cystic corpora lutea were found in pregnant cows. Cows with cystic corpora lutea often have estrous cycles of normal length, and this condition may be a more important cause of infertility, especially that due to early embryonic mortality, than has been generally recognized.

Foley and Greenstein (67) classified the cells of the bovine corpus luteum into five types accurately identified by cytological characteristics. Type I is described as an immature cell, while Type II cells have reached their maximum size and development. Types III, IV, and V represent progressive stages of regression. Attempts are being made to relate the percentages of these various cell types to the previous breeding history of animals from which the corpora lutea are obtained.

The color of the bovine corpus luteum is described as changing from brown to bright yellow between the 5th and 14th days of the cycle, after which it becomes orange by about the 20th day (79, 109, 126). During involution it becomes a brick-red color.

C. Changes in the Oviduct

Although the bovine oviducts have been studied by several investigators (114, 150, 167, 187), less is probably known about how they function than for any other part of the bovine reproductive tract.

The wall of the oviduct consists of an outer serous coat, a muscular layer made up of longitudinal and circular unstriated muscle fibers, and an inner mucosal layer. The ovarian region, or fimbriated portion of the oviduct, has thin walls and the mucosa is thrown into many projecting folds. In the midportion, the number and height of the folds is decreased and the thickness of the muscle layer increased. The uterine portion has a very thick muscular wall and in cross section resembles a miniature uterus.

The mucosa at the ovarian end is almost entirely covered with cilia. The number of these cilia decreases as the uterus is approached. Extruded nuclei are common and appear to arise from the epithelial cells. They are most numerous in the ovarian end of the oviducts (Fig. 2).

The epithelium of the fimbriated ends of the oviducts reaches its maximum height (35-47 μ) during and shortly after estrus, and is lowest (21-38 μ) at 8-9 days postestrus (150). In the late luteal phase the epithelium ranges from 28-32 μ . The cilia, which beat toward the uterus, are 7-10 μ in length during estrus and 5-8 μ at other stages of

the cycle. The extrusion of nuclei into cytoplasmic projections is maximal at about 9 days postestrus. The changes in the mucosal epithelium of the middle portion of the oviduct are similar to those in the fimbriated segment. Cyclic changes have not been noted in the uterine segment of the oviduct.

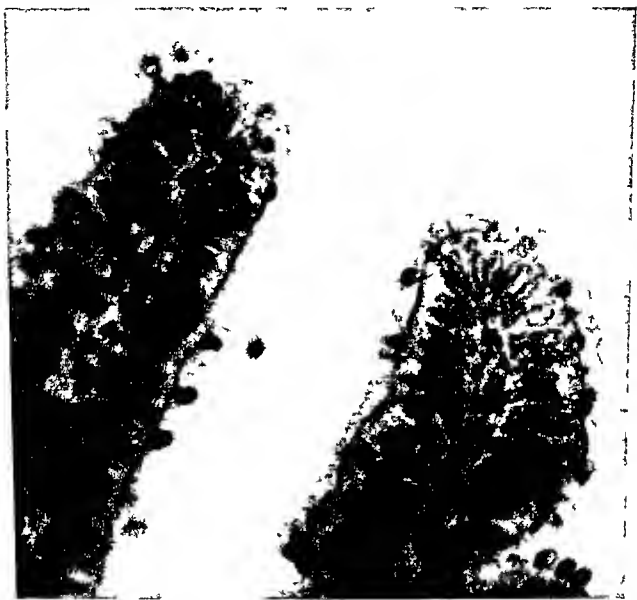


FIG. 2. Mucosa of the ovarian end of the oviduct of a heifer slaughtered at the 21st day of the estrous cycle and prior to the beginning of estrus. Note the extruded nuclei. Magnification $\times 550$.

D. Changes in the Uterus

The wall of the bovine uterus consists of a thin outer layer (the perimetrium), a thick muscular layer (the myometrium), composed of an inner circular and an outer longitudinal smooth muscle layer, and an inner lining (the endometrium).

1. Changes in the Endometrium

The endometrium consists of connective tissue elements (stroma), in which the uterine glands are interspersed, and the epithelial lining, which covers the luminal surface of the uterus. The uterine glands are branched, coiled, and tubular, and are lined with columnar epithelium (Fig. 3). They open on the surface of the endometrium, except in the caruncular areas. The stroma beneath the epithelial layer consists of a narrow layer of dense connective tissue and a deeper layer of loose connective tissue. The caruncles are composed of dense connective tissue with numerous coiled arterioles, and are covered by the epithelial layer.

The endometrium in young heifers is shallow and contains few glands. As sexual maturity is approached, the endometrium thickens and the glands become more numerous. At the time of first estrus, the surface epithelium increases from about 14 to 36 μ and the glandular epithelium from 18 to 27 μ (167).

There is general agreement that the endometrial surface epithelial cells are tall during proestrus and estrus. As a result of active secretion during and shortly after estrus, they become low and cuboidal by 2 days postestrus (7, 52, 150, 186, 187). Following the formation of the corpus luteum, the height of these cells increases, reaching a maximum at the 9th to 12th day of the cycle. The nuclei are elongated and basally situated during estrus, and become oval by the 9th day of the cycle.

Moss *et al.* (133), Weeth and Herman (187) and Skjerven (165) studied the alkaline phosphatase activity of the surface epithelial cells. The highest activity was found during mid-cycle. Little or no phosphatase activity was present at the beginning and end of the cycle. The alkaline phosphatase activity thus parallels the activity of the corpus luteum. Glycogen distribution in the surface epithelium also showed a distinct cyclic variation, being present in largest amounts at proestrus and estrus when follicle growth is maximal. Glycogen seems to accumulate when phosphatase activity is low or absent. Fat was noted in the surface epithelium between 10 days postestrus and 2 days proestrus.

All authors agree that the uterine glands are relatively straight at the time of estrus (7, 52, 150). They begin to grow, secrete, and become more coiled at 2 days postestrus, and increase in complexity and secretory activity until the 12th day of the cycle. The glandular epithelium reaches its maximum height at the 8th day. Retrogression of this glandular hypertrophy has been observed as early as the 15th day. No distinct cyclic variation in either alkaline phosphatase activity or glycogen distribution has been noted in the glandular epithelium (133,

165), but alkaline phosphatase is always present in the fibrous sheaths of the glands

Vascular congestion and edema are observable in the stroma several days before the onset of estrus. By the beginning of estrus the stroma has become extremely edematous, thus the cells and connective tissue



FIG. 3 Endometrial glands in the uterus of a heifer at the 11th day of her 11th estrous cycle. Magnification $\times 27$ (52)

elements become widely separated (7, 52, 57, 150). The vascular congestion and edema subside by the second day after estrus, and hemorrhage beneath the surface epithelium and erythrocytes in the surface epithelium, indications of metestrus bleeding, may be observed at this time (86, 186). The vascularity and congestion of the superficial stroma is increased again at the 8th to 10th day of the cycle, but very little extravasation occurs at this time.

Skjerven (165) found neutrophils in the superficial stroma and surface epithelium a few days before, during, and after estrus, but found practically none at other stages of the cycle. The number of eosinophils in the stroma was quite variable and unrelated to the stage of the cycle. The number of plasma cells and mast cells in the stroma increased with advancing age of the animal. Mast cells were always present in an area 75-100 μ beneath the surface epithelium; the highest number was present during the follicular phase of the cycle.

The cow differs from other species studied in that large amounts of alkaline phosphatase are found in the endometrial connective tissue stroma. The amount of phosphatase activity in the stroma increases directly with the increase in density of the stromal cells and fibers, and shows cyclic variation only to the extent of fiber density variation within the cycle (133). The presence of phosphatase activity in the stromal fibers of the uterus seems to depend on an association with the endometrial glands. Glycogen is found in the upper endometrial stroma, either in a free state or in small, round cells, from shortly after estrus until the 14th day of the cycle, at which time Skjerven (165) reported that it disappears. This disappearance of stromal glycogen corresponds to its reappearance in the surface epithelium.

2. *Changes in the Myometrium*

Cupps and Asdell (55) studied the length of the cells in the myometrium of the cow at various stages of the cycle and report that they grow in length during the estrogenic phase and decrease during the remainder of the cycle. The growth impulse appeared to start at the apices of the uterine horns and traveled caudally.

The uterine arteries supplying the endometrium and myometrium in the cow were studied in some detail by Hansel and Asdell (83). The arterioles serving the caruncles are quite coiled. The endometrial arterioles in 13-16-month-old heifers are essentially straight, while similar arterioles in mature cows are more numerous and more highly coiled. Differences in the endometrial arterioles associated with the estrous cycle could not be detected. The longitudinal muscle layer is supplied by small, T-shaped, coiled arterioles.

E Changes in the Cervix

The changes in the mucosa of the cervix during the cycle have been described in detail by Cole (52), Rorick and Herman (150), Hammond (79), and Herriek (99). The epithelium of the external annular fold, which is fairly representative of that in other areas of the cervix, is 2 or 3 cells deep during proestrus. The superficial layer at this time consists of wide goblet cells between which compressed nuclei are often seen. In the body of the glands only a single layer of cells occurs, some of these are narrow, indicating that mucous secretion has already started. The stroma is loose, cell-poor, and slightly edematous at this time.

During estrus the epithelial cells are tallest (5-24 μ), and their nuclei are arranged with their long axes perpendicular to the basal membrane. Mucus is present in the deep cervical glands and secretion is active (Fig. 4).

At 2 days postestrus the height of the superficial layer is reduced in many areas, and the nuclei are either oval or flattened. The stroma has become more dense.

At 8 to 11 days postestrus the epithelium is composed of a single layer of low columnar cells with oval, basal nuclei. The epithelium takes on a ragged appearance and the cells no longer contain mucus. The stroma is dense and shows no sign of edema.

F Changes in the Vagina

Mucous secretion begins in the cervix during proestrus and progresses toward the posterior portion of the vagina where mucus-secreting cells are observed for the first time at 2 days postestrus (52). The changes occurring in the mucosa of the anterior vagina during the cycle resemble those in the cervix. Large, mucus-secreting cells make up the superficial epithelium during proestrus and estrus. The layers of cells in this area are reduced from 3 or 4 during proestrus to 1 or 2 during estrus, and are increased to 3 or 4 again by 2 days postestrus. At 2 days postestrus the cells are enboidal and ragged and at 8 to 11 days postestrus the epithelium is vacuolated and appears degenerate in character (6, 8, 52, 79, 150). Desquamation has not been observed in this portion of the vagina.

The vaginal epithelium near the urethra is irregular in depth throughout the cycle, varying from 4 to 33 cell layers. During proestrus (Fig. 5) the epithelium consists of small, compact cells and small epithelial follicles filled with lymphocytes are frequent. The superficial cells are polyhedral rather than squamous in type. During estrus the average epithelial cell height increases to as much as 54 μ , as compared to the

46 μ found during proestrus. The stratum germinativum becomes more pronounced and the nuclei have their long axes perpendicular to the membrana propria. Lymphocytes and leucocytes are present in all epithelial layers.

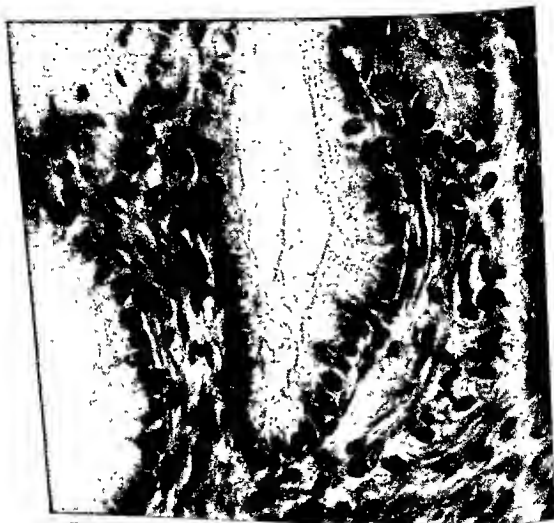


FIG. 4. Glandular epithelium in the cervix of a heifer slaughtered at the 21st day of the estrous cycle and prior to the beginning of estrus. Magnification: $\times 550$.

At 2 days postestrus the epithelial cell height increases markedly to about 81 μ . The concentration of lymphocytes and leucocytes is reduced. At 10 days postestrus the superficial layers of epithelium tend to become squamous, but true cornification does not occur.

Since the anterior vagina consists largely of mucus-secreting cells, and true cornification does not occur, it is not surprising to find that the vaginal smear of the cow cannot be used to diagnose accurately the stage

of the cycle in the cow as in other species (52, 79, 82, 134, 198). Hansel *et al.* (82) described vaginal smears aspirated from the cervical end of the vagina during estrus as containing many leucocytes, some large, round epithelial cells, and very few cornified cells. At one day postestrus the smear consisted largely of round, nucleated epithelial cells. Few leucocytes and few cornified cells were present in the smear from the 2nd to the 9th day. At about the 9th day the percentage of cornified cells

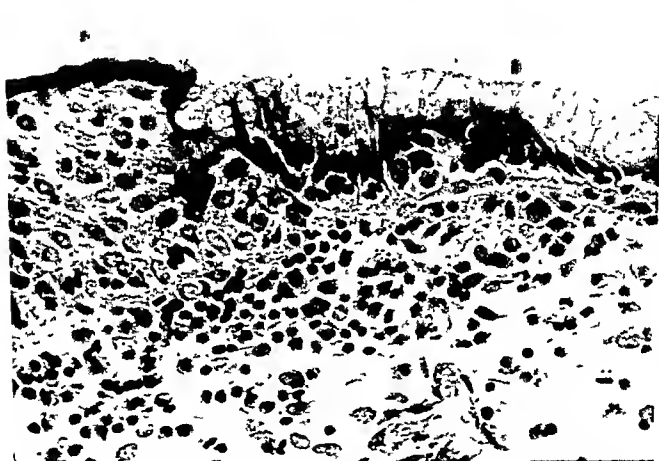


FIG 5 Cross section showing the epithelium of the anterior vagina of a heifer slaughtered at the 21st day of the estrous cycle and prior to the beginning of estrus. Magnification $\times 552$ (52).

and the number of leucocytes in the smear rose and remained elevated until the 16th day. The percentage of cornified cells seen rarely exceeded 30% of the cells in the smear at any time during the cycle. The percentage of cornified cells found in smears taken during proestrus was variable, but tended to be lower than in smears taken between the 9th and 16th days. Leucocytes, however, were even more numerous during this period and many small, round, nucleated epithelial cells were seen. There was considerable individual variation as to the extent and exact time during the cycle at which these changes occurred.

G. Vaginal and Cervical Secretions

The physical and chemical properties of the vaginal and cervical secretions of the bovine have been studied in some detail. Scott Blair *et al.* (22, 23, 24) and Roark and Herman (150) studied the flow-elasticity of cervical mucus in an instrument designed to measure the recoil of a sample of mucus in a capillary tube after pressure was released. They report that recoil is maximal at the beginning of estrus and declines during the estrous period. During diestrus the cervical mucus shows very little flow-elasticity. The volume of mucus, its water content, and surface tension decline from the beginning to the end of estrus. The nitrogen content of mucus on a dry matter basis is low at estrus (1-2%) and rises (6-10%) at mid-cycle. Scott Blair (24) and Glover (72) also studied the "consistency," flow-birefringence, and infrared and ultraviolet absorption spectra of cervical mucus. An ultraviolet absorption peak at 278 m μ was found in mucus from pregnant cows and ovariectomized cows treated with estrogen-progesterone but was hardly measurable in cows in estrus or estrogen-treated ovariectomized cows. A characteristic infrared absorption band found only at the end of estrus and a few days afterward was also reported.

A fernlike pattern formed by cervical mucus on drying that is characteristic of estrus or high estrogenic activity has been reported for the bovine (24, 25, 71). The fern pattern occurred in cervical mucus obtained from about 3 days proestrus to about 9 days postestrus. It did not occur at times during the cycle when the activity of the corpus luteum was maximal, or during pregnancy. Interestingly, in the study of Bone (25), the fernlike pattern was formed in cervical mucus from 13 cows with cystic corpora lutea, suggesting either a subnormal output of progesterone or the production of estrogens by these abnormal corpora lutea. This test apparently is not sufficiently accurate to be useful as a test for pregnancy. It has not been studied sufficiently to test its usefulness as an indicator of the relative amounts of estrogen and progesterone being secreted during the cycle.

The pH of vaginal mucus, both *in vivo* and *in vitro*, has been exhaustively studied (see review by Roark and Herman, 150). The pH of the vaginal secretions *in vivo* is lower (6.57) than the pH of the same secretions after they are removed from the tract (7.45). Both *in vivo* and *in vitro* determinations show that the lowest pH occurs in early estrus, the mucus becoming more alkaline as estrus progresses. There do not appear to be any other marked changes in pH associated with the cycle. Cervical mucus is slightly more acid than vaginal mucus. There is little if any relation between pH of the vaginal secretions and fertility.

The chemical composition of vaginal mucus has not been studied extensively. It appears to contain a mucopolysaccharide-protein complex (29, 129). Roark and Herman (150) obtained color reactions for bovine mucus characteristic of glycogen, peptide linkages, and the amino acids tyrosine, cystine, tryptophan, and phenylalanine.

Olds and Van Demark (142) reported the composition of vaginal mucus obtained from the cervical area to have an average dry matter content of 24%, of which 42.6% was ash and 18.0% ether extractable material. The total nitrogen content was 0.18 g per 100 ml and reducing sugars amounted to 161 mg per 100 ml. The following average concentrations (mg per 100 ml) were found: Na, 170; K, 166; Ca, 11.3; P, 1.5; and Cl (as NaCl), 526. The data, although based on limited numbers, indicated an increase in potassium concentration of the vaginal mucus during the luteal phase of the cycle, and an increased calcium concentration near estrus.

H Uterine and Oviduct Fluids

Olds and Van Demark (141) also studied bovine uterine and oviduct fluids. Uterine fluid during luteal stages contained 400 million epithelial cell nuclei per milliliter. The dry matter amounted to 8.4% of the uterine fluid and 13.6% of the oviduct fluid. The ash constituted 19.6% of the dry matter of the uterine fluid and 7.52% of the dry matter of the oviduct fluid. The ether extract amounted to 1.4% of the dry matter of the uterine fluid and 13.5% of the dry matter of the oviduct fluid. Reducing sugars amounted to 78.4 and 89.2 mg per 100 ml for uterine and oviduct fluids, respectively. The following ion concentrations (in mg per 100 ml) were found in uterine fluids: Na, 220; K, 183; Ca, 15.2; P, 7.4; and Cl (as NaCl), 362. Comparable figures for oviduct fluids were: Na, 208; K, 223; Ca, 11.8; P, 9.7; and Cl (as NaCl), 400. Sodium levels were much higher in both uterine and oviduct fluids during the luteal stages of the cycle. The potassium level in oviduct fluids and the calcium level in uterine fluids were elevated at estrus. Average pH values were 7.8 for vaginal mucus, 7.1 for uterine fluids, and 6.4 for oviduct fluids.

The same authors (Olds and Van Demark, 141) have recently prepared an extensive review of the literature on fluids in the bovine genital tract.

IV EFFECTS OF VARIOUS HORMONES ON THE REPRODUCTIVE TRACT

Most of the changes described in the various parts of the bovine reproductive tract are brought about by the ovarian hormones secreted

in varying amounts during the cycle. Presumably, the theca interna cells secrete the estragenic hormone, estradiol, in increasing amounts during the development of the follicle. Estradiol may later be converted into less active estragenic substances, estrone and estriol, in the liver and other tissues. After ovulation the corpus luteum forms and secretes progesterone in increasing amounts until about the 15th day of the cycle.

These steroid hormones cooperate to produce the remarkably coordinated series of events just described for the reproductive tract. All of these modifications are designed to bring the ovum and the sperm together for fertilization and to prepare the uterus to receive and nourish the growing embryo. Hormones other than estrogen and progesterone also cooperate in bringing about changes in the reproductive tract; these will be considered separately.

A. Estrogen and Progesterone

Estrus can be produced in the ovariectomized cow by remarkably small doses of estrogenic substances (5, 127, 169). Melampy *et al.* (127) reported that small amounts of progesterone can act synergistically with estrogens in the production of estrus. Larger doses of progesterone, on the other hand, clearly inhibit the induction of estrus in ovariectomized cows and reduce its length in normal cows (81, 127). In addition to producing estrus in ovariectomized cows, estrogenic hormones have been shown to cause many of the changes in the reproductive tract occurring in the normal animal during the estrous cycle.

1. Effects on the Uterus

Asdell *et al.* (7) found that estrogen injections up to 1500 I.U. per day into ovariectomized heifers increased the height of the endometrial epithelium, produced marked stromal edema, and increased the size of the lumina of the endometrial glands. The development and degree of coiling characteristic of endometrial glands at the 12th day of the normal cycle were not produced by estrogen alone, but daily injections of 300 I.U. of estrogen and 18 or 33 mg. of progesterone for 6 days produced the endometrial development characteristic of this stage of the normal cycle.

Sykes *et al.* (169) showed that the surface epithelium and dense endometrium appreciably decreases in phosphatase concentration after ovariectomy. The subcutaneous administration of 0.6 mg. of estradiol benzoate daily for 3 days produced estrus and an increased concentration of phosphatase in the surface epithelium similar to that seen at mid-

cycle in normal cows The concentration of phosphatase in the dense upper endometrial stroma remained rather low after estrogen treatment The injection of 40-60 mg of progesterone per day for 5 days effectively increased the phosphatase in the endometrial stroma, especially in the dense endometrium The glycogen content of the surface epithelium remained high after ovariectomy and disappeared after estrogen injections Glycogen was present in the surface epithelium after progesterone injections It is interesting to note that estrogen injections produced a pattern of phosphatase and glycogen distribution most typical of the mid cycle in the normal cow, and progesterone produced a pattern most typical of metestrus

The spontaneous motility of the uterine musculature during the cycle is also regulated to a large extent by the ovarian hormones Evans and Miller (65) reported marked spontaneous motility of the uterus from 1 day proestrus to 1 day postestrus, after which the motility declined until the 16th day, and then increased until estrus Cupps and Asdell (55) reported similar results after studying the spontaneous activity of excised longitudinal uterine muscle strips, but Hays and Van Demark (97), using the intra uterine balloon technique, reported regular and frequent contractions of small amplitude during estrus and less frequent contractions of greater amplitude in mid cycle These workers found no significant variations during the cycle when the product of the frequency and the amplitude of contraction was used as an index of activity There is agreement that ovariectomy causes a marked decline in uterine motility and that estrogen injections increase uterine motility in ovariectomized cows (5, 97) Although the frequency of contractions was lower than in the normal uterus, injections of progesterone after ovariectomy caused an increase in the amplitude of contractions (Hays and Van Demark, 97) Asdell *et al* (5) also reported contractions of great amplitude in uterine muscle from ovariectomized progesterone treated heifers

Some of the major functions of the estrogenic hormones in the cow, as in other species, is to cause uterine hyperemia, edema, and endometrial growth Estrogens appear to be responsible for the development and maintenance of the endometrial arterioles in the cow The number and the degree of coiling of the endometrial arterioles was found to be much greater in mature cows than in young heifers, and these arterioles largely disappeared after ovariectomy (83) Estrogens, supplemented by relatively small amounts of progesterone, were effective in preventing the postcastration atrophy of the endometrial arterioles

The bovine uterus is more resistant to bacterial infection during

estrus than during other stages of the cycle (17, 19, 21, 111). This difference has been attributed to a reduction in resistance brought about by progesterone (20), but estrgens also may increase uterine resistance to infection (111, 154, 155), perhaps indirectly by increasing the blood supply to the uterus and the flow of mucus through the cervix.

2. *Effects on the Cervix*

Changes in the cervix characteristic of those at estrus have been produced by the ovarian hormones. Estrogen injections increase the height of the epithelial cells and mucous secretion (8, 42). The effects of estrogen and progesterone on the physical and chemical characteristics of cervical mucus were described earlier in connection with the cyclic changes in the vaginal and cervical secretions.

3. *Effects on the Vagina*

The vaginal smears from ovariectomized heifers brought into estrus with stilbestrol are quite similar to the smears obtained from normal heifers at the time of estrus, in both instances consisting of numerous leucocytes, a few large epithelial cells, and very few cornified cells (82). Large numbers of round, nucleated epithelial cells appear in the smear 2 days after the stilbestrol-induced estrus, as they do in the smear of the normal animal at 2 days postestrus. Subsequent to the second day after estrus the percentage of the cornified cells in the vaginal smears of the stilbestrol-treated heifers rises; this increase is not inhibited by progesterone injections.

B. *Effects of Other Hormones on the Bovine Reproductive Tract*

Relaxin is extractable from ovaries of pregnant cows (81). Graham and Dracy (75) observed that it caused relaxation of the cervix when administered at 5 days postestrus to normal cows previously treated for 3 days with diethylstilbestrol. Stilbestrol alone did not cause cervical relaxation, although the cervix is normally dilated at the time of estrus.

Most evidence indicates that oxytocin causes uterine contractions in the cow at all stages of the estrous cycle (55, 65, 96) and Asdell *et al.* (5) and Hays and Van Demark (96) have reported that stilbestrol treatment increases the response of the uterine musculature of the ovariectomized cow to oxytocin. Asdell *et al.* (5) reported strong and Van Demark and Hays (96, 183) reported weak contractions in response to oxytocin by uterine muscles of ovariectomized progesterone-treated cows.

Cupps and Asdell (55) found that epinephrine inhibits uterine contractions during and shortly after the estrogenic phase of the estrous

cycle and causes contractions during the progestational phase Asdell *et al* (5), using an *in vitro* method, also reported relaxation of the uterine musculature of the ovariectomized estrogen-treated cow in response to epinephrine, as contrasted to contraction in the ovariectomized progesterone-treated cow Uterine muscle from ovariectomized cows receiving 500 IU of estradiol benzoate and 18 mg of progesterone daily for 6 days showed a diphasic response to epinephrine, i.e., a contraction followed by relaxation Hays and Van Demark (96, 183), on the other hand, reported that epinephrine *in vitro* and *in vivo* caused one large contraction followed by a period of reduced activity and tone in normal cows, in an ovariectomized stilbestrol treated, and in an ovariectomized progesterone treated cow Epinephrine, given before oxytocin, completely or partially inhibited the increased activity in response to oxytocin Alexander (2) reports epinephrine inhibition of uterine motility in both pregnant and nonpregnant cows

V CHANGES IN OTHER ENDOCRINE GLANDS DURING THE ESTROUS CYCLE

A The Anterior Pituitary

The literature concerning changes in the bovine pituitary gland associated with the estrous cycle was reviewed in 1955 by Jubb and McEntee (105), the present discussion will be confined to recent findings on this subject Jubb and McEntee (106) were able to enlarge our knowledge on the functional cytology of the bovine anterior pituitary considerably by utilizing the periodic acid Schiff (PAS) staining technique The large basophils (beta cells) concentrated in the medulla of the gland contain PAS-staining granules, these cells probably produce thyrotropin, since they become enlarged, degranulated, and vacuolated after thyroidectomy

The small basophils (delta cells) contain coarse PAS-staining granules and usually are found in close association with the blood sinusoids These cells are probably gonadotropin producers, and they degranulate rapidly within a matter of hours as a cow comes into estrus (Fig 6) The degranulation process begins in the medulla adjacent to the zona tuberalis and sweeps on a broad front through the whole pars distalis proper A recent study (89) indicates that this degranulation process may be essentially complete as early as 20 minutes after a cow comes into standing estrus This delta cell degranulation process does not occur in cows which come in estrus and later fail to ovulate

The granules in the delta cells begin to reappear about 3 days post-estrus, and then accumulate rapidly during the luteal phase of the cycle, reaching a maximum during proestrus The delta cell granules

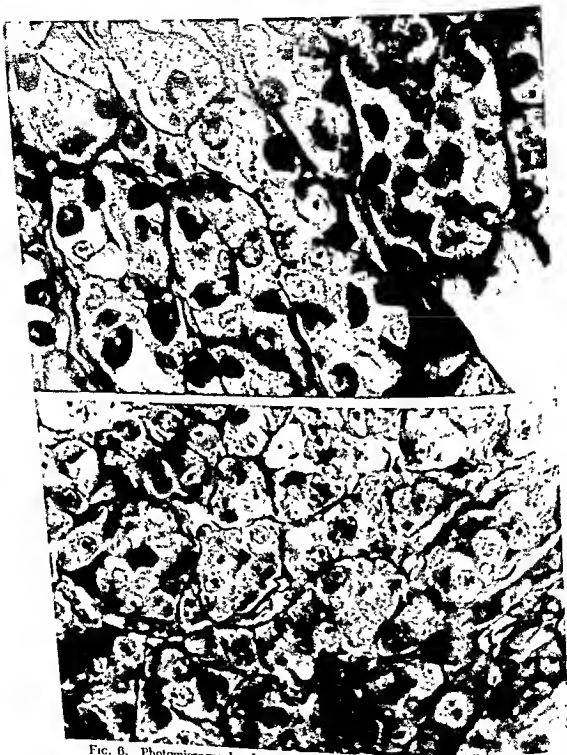


FIG. 6. Photomicrographs showing the heavy granulation of the pituitary delta cells in a heifer slaughtered in late proestrus (top) and the nearly complete denigration of these same cells in a heifer slaughtered early in estrus (PAS). Mag: $\times 345$. (Courtesy of K. V. Jubb, Ontario Veterinary College, Guelph, Ontario.)

disappear in steers and in cows having ovarian cysts for long periods of time. Regranulation of the delta cells appears to occur in steers fed stilbestrol (91). Delta cells are also found in the pars tuberalis and the zona tuberalis, but they have not been observed to undergo cyclic changes.

Changes in the acidophils occur later during the cycle and are less abrupt. These cells begin to degranulate at the 3rd day after estrus and reach a maximum of degranulation at the 10th day. During the remainder of the cycle the acidophils accumulate granules. These changes parallel the activity of the corpus luteum, suggesting that the acidophils are the source of prolactin. In addition, they probably produce the growth hormone, but it has not been possible to subdivide them into specific types producing each hormone. The acidophils are hypergranulated and have small, crenated nuclei in cows with cystic ovaries.

It is not known definitely which gonadotropin is released during the delta cell degranulation process, nor has a specific type of cell been identified for each gonadotropin. Hopes that the delta cell degranulation process could be specifically linked to LH release have been dimmed somewhat by the finding that atropine injections early in estrus block ovulation, and presumably LH release, even in cases where the delta cell degranulation has occurred (89).

Cupps *et al* (56) recently reported that the percentages of beta cells (which apparently correspond to the delta cells described above) range from 24 to 34 in the medullary zone of pituitaries of normal cattle. The percentage of these cells was increased in nymphomaniac cows in which the ovaries contained no luteal tissue.

B The Adrenals and Thyroid

Specific changes in the size and histology of the adrenal glands of cattle throughout the estrous cycle do not appear to have been described, but there are several reports of changes in the adrenals related to ovarian function. Bourne and Zuckerman (28) showed that adrenal weights and volumes in rats increase during estrus, largely due to an increase in size of the cells in the zona fasciculata. Garm (69) noted that sudanophilic substances and substances showing birefringence are absent or sparse in the glomerulosa of normal cows at the time of estrus and in nymphomaniac cows. This finding suggests a relationship between the circulating estrogen level and the depletion of corticosteroids produced in this zone. Cupps *et al* (56) found evidence of adrenal degeneration in cows with irregular estrous cycles, and these authors, and Garm (70) reported adrenal hypertrophy in nymphomaniac cows.

There are no reports of changes of a regular nature in the thyroid associated with the estrus cycle in the cow. The thyroid glands of rats undergo cyclic changes, being most active in terms of oxygen consumption and iodine uptake during estrus (148). Thyroidectomized cows did not show the physical manifestations of estrus (33, 168), but the ovaries continued to function in an apparently normal manner.

VI. METHODS OF ALTERING THE CYCLE

A great deal of experimental work has been conducted in an attempt to find a simple and dependable method for regulating estrus and ovulation in the cow, without at the same time reducing fertility. There are several potential practical applications for such a method in both beef and dairy cattle operations. In addition to providing a useful treatment for several types of infertility, a method for producing estrus and ovulation at a predetermined time might allow large numbers of animals to be bred within a period of a few days. A development of this sort will probably be necessary before artificial insemination is used on a large scale in the beef cattle industry.

A. Corpus Luteum Removal

Perhaps the simplest method for altering the bovine estrous cycle consists of manual removal of the corpus luteum through the rectal wall. Numerous investigators have studied the effects of corpus luteum removal. Roberts (151) has summarized the results of these studies by stating that observable estrus results in 50-80% of the cases treated within 2 to 7 days and that 50-55% of these cows conceive if bred.

Regardless of the skill of the person performing the operation, an occasional cow will die after corpus luteum removal as a result of excessive hemorrhage. The incidence of adhesions in and around the oviducts also appears to be increased as a result of corpus luteum removal, a factor which may lead to infertility in subsequent years. These factors have led to a decline in the popularity of manual corpus luteum removal as a treatment for "retained" corpora lutea and almost preclude the routine use of this method to regulate estrus and ovulation. Some workers feel that the corpus luteum is never really retained unless excess fluid or some foreign substance is present in the uterus.

B. Progesterone Injections

Most of the experiments concerning estrous regulation have involved the daily injection of large (50-100 mg.) doses of progesterone from mid-cycle onward. All authors are in agreement that this treatment

prevents estrus and ovulation from occurring at the normal time, and that estrus and ovulation do occur 4-7 days after cessation of the treatment (49, 174, 177, 178, 179, 188). Nellor and Cole (135) found that single injections of 540-1120 mg. of crystalline progesterone prevented estrus and ovulation in beef heifers, regardless of the stage of the cycle when the treatment was given. Estrus occurred 15-19 days after the progesterone injection in 89% of the heifers receiving 540-560 mg. of progesterone. Trimberger and Hansel (174) obtained only a 12.5% conception rate at the first post treatment service after daily progesterone injections of 50-100 mg. were used to control estrus. The cycles following the post treatment estrus were of normal length and a normal conception rate (65.2%) was obtained in cows bred after these cycles. Nellor and Cole (135) also found a low conception rate (17%) in beef heifers injected with 500 mg. of crystalline progesterone, followed 15 days later by a single injection of equine gonadotropin. The same authors (Nellor and Cole, 136) reported that injections of amounts of progesterone capable of inhibiting follicle growth, estrus, and ovulation in beef heifers had no detectable effect on the amounts of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH) or somatotropin (STH) contained in the anterior pituitary glands.

These reports indicate that it is possible to regulate estrus and ovulation by progesterone, or progesterone and gonadotropin injections, but that fertility is lowered by these treatments. It is not yet clear whether this infertility is a result of degenerative changes in the ovum associated with the delay in ovulation (32) or a consequence of the too rapid transport of the ovum through the oviduct in the presence of progesterone (153). Both factors may be involved.

C. Gonadotropin Injections

Because human chorionic gonadotropin has many physiological properties in common with pituitary LH, no attempt will be made to differentiate the two in the discussion that follows in this chapter.

Many of the studies illustrating the effects of gonadotropins on ovarian function in the bovine were carried out for the purpose of producing "superovulation," i.e., the release of many ova at one time. The problems involved in superovulation and ovum transfer have recently been reviewed by Willett (193), and need not be considered here, except as they relate to the basic effects of the gonadotropins on the estrous cycle.

Results obtained after the injection of gonadotropic preparations into

calves show that the ovary of the calf is capable of responding to gonadotropin with follicle growth almost from the time of birth (18, 39, 117, 118). Despite this marked follicular growth, only about 50% of calves treated with a series of FSH injections, followed by an intravenous injection of an LH-containing preparation, formed corpora lutea. Marden (117) obtained a higher proportion of ovulations in response to a second series of gonadotropin injections after a period of luteal activity, but Black *et al.* (18) were unable to achieve a similar result by pretreatment with progesterone. Estrus did not accompany ovulations produced in calves by these treatments, and the percentages of eggs fertilized were very low.

The various methods used to produce superovulation in mature cows have been discussed by Willett (190). The effects of exogenous gonadotropins on ovarian function in mature cows are dependent upon the stage of the cycle at which the treatments are given, and whether or not the corpus luteum is removed prior to or during the treatment. The subcutaneous injection of single or multiple doses of pregnant mare serum (PMS) as a source of FSH, followed by an intravenous injection of chorionic gonadotropin as a source of LH during the first half of the estrous cycle while a functional corpus luteum is present results in the production of numerous follicles, a high percentage of which ovulate (32, 39, 40, 59, 110, 153, 180). Rowson (153) found that 52% of the follicles ovulated in cows whose ovaries contained a large corpus luteum at the time of intravenous LH injection, in contrast to a 14% ovulation rate in cows whose ovaries contained no corpora lutea. A similar high ovulation rate was obtained in PMS-treated cows in which the corpora lutea were removed and 20 mg. of progesterone was injected daily for 4 days prior to the LH injection. Although numerous ova are shed when the combined PMS and LH treatment is given during the first half of the cycle, they are not capable of being fertilized (32, 43, 110, 153, 180). Some, but by no means all, of the cows treated in this way come in estrus, and the intravenous LH injection has usually been given at the time of estrus or 5 days after the FSH injection in cases where estrus did not occur.

Single or multiple injections of FSH in the latter part of the cycle result in the presence of an increased number of large follicles at the time of estrus, but the percentage of these follicles that ovulate either with or without an injection of LH at the time of estrus is relatively small (32, 39, 59, 80, 153, 189). A fairly high percentage of ova produced in this way are fertile (32, 59, 189, 191).

The injection of a source of FSH at mid-cycle immediately following

removal of the corpus luteum, and the injection of LH during the estrus which ensues in 2 to 4 days appears to be the best method investigated to date for the production of a maximum number of fertile ova (32, 54, 80, 110, 153, 189) Brock and Rowson (32) have used doses of 3000 I U of whole PMS followed by 2000 I U of LH at the time of estrus for this method, and report ovulation rates of 12.5% in cases where LH was not given at estrus, in contrast to 22.3% in cases where it was given

High doses of PMS have generally produced large unovulated follicles (59, 68, 189) Willett *et al* (192) have demonstrated a decreasing number of corpora lutea in cows which were subjected to successive superovulations. The development of this refractoriness may be due to the formation of antigonadotropins

Marion and Smith (120) found that unfractionated pituitary gonadotropic extracts given early in estrus hastened ovulation, and Hansel and Trimberger (84) reported that chorionic gonadotropin has a similar effect, even in heifers given the ovulation-blocking drug, atropine, at the beginning of estrus. Smith *et al* (166) have recently reported that daily doses of 300 to 1800 I U of prolactin are incapable of maintaining the size of the corpus luteum or prolonging the estrous cycle in the cow

D Oxytocin Injections

Daily injections of oxytocin beginning before ovulation occurs have recently been found to alter the bovine estrous cycle (3). These results are discussed in more detail in the section concerning the mechanism of ovulation

VII OVARIAN HORMONE LEVELS IN BLOOD AND EXCRETA DURING THE ESTROUS CYCLE

A Estrogenic Substances

Relatively little is known of the levels of estrogens, or their metabolites in the blood, urine, or feces of the cow during the normal estrous cycle. Woods (196) found evidence that the phenolic steroids in pregnant cow's urine are estradiol and estrone. Estriol was not found. Turner *et al* (176) reported an average of 57 rat units of estrogenic activity per day in the urine of nonpregnant cows, but Asdell *et al* (5) were not able to find measurable amounts. Gorski *et al* (74) were successful in assaying the estrogens in acid-hydrolyzed urine from nonpregnant cows by the 6-hour immature rat uterine weight method, and obtained estimates of 73–173/ μ g of estrone equivalents per day. Urine of two cows at 8 days proestrus appeared to contain about as much

estrogenic activity as the urine of a cow in estrus. The urine of an ovariectomized cow contained little activity.

Szego and Roberts (170) reported approximately 0.3/ μ g. of estradiol per 100 ml. of whole blood from a nonpregnant gonadotropin-treated cow, but Bitman and Sykes (16) have been unable to consistently measure estrogens in the blood of nonpregnant cows.

B. Progesterone

Cow's urine does not appear to contain pregnanediol (100), despite the earlier reports summarized by Cole (53) to the contrary. Edgar (62) devised a method for the chemical assay of progesterone in body fluids and reported (61) the presence of progesterone in bovine follicular fluid prior to the time of ovulation, and its absence in developing follicles. Raeside and Turner (146), using a similar method, were unable to detect progesterone in the peripheral blood of nonpregnant cows. Injected progesterone disappears rather quickly from the peripheral blood of the cow (146), as it does in other species (94). Progesterone is present in ovarian vein blood from ewes (63), goats (146), and, probably, cows (91) when a corpus luteum is present in the ovary.

VIII. THE MECHANISM OF OVULATION IN THE COW

The alterations in the bovine estrous cycle previously discussed have all been brought about by injections of the ovarian or gonadotropic hormones or combinations of these hormones, and have been explained in terms of generally accepted interactions between these hormones.

The growth of a follicle in the ovary in the cow, as in other species, appears to be brought about by FSH secreted by the anterior lobe of the pituitary gland. A very small amount of LH may facilitate the process of follicle growth, but the rupture of the matured follicle and the formation of the corpus luteum require the release of a much larger quantity of LH from the anterior pituitary. The production of progesterone by the corpus luteum in the cow, as in other species, has been assumed to be controlled by prolactin from the anterior pituitary. In addition to these effects of the three gonadotropic hormones, it has been generally accepted (6) that estrogens facilitate ovulation by depressing FSH secretion by the anterior pituitary and by increasing LH secretion. It has also been assumed that progesterone inhibits LH secretion and ovulation.

This concept of the mechanism of ovulation explains many of the experimental results that have been obtained in studies involving estrus and ovulation in the cow, but there are certain experimentally estab-

lished facts that it can not encompass. Estradiol administered to the cow at the beginning of estrus does not hasten ovulation, as might be expected in view of the above concept (85), in fact, it delays ovulation in the ewe (60). Progesterone does not always inhibit ovulation; under certain conditions it is ovulatory in the cow, as it is in many other species (87). It is not possible to prolong the estrous cycle in the cow by daily injections of as much as 1800 I.U. of prolactin during the latter third of the estrous cycle (166), even though daily injections of progesterone during this period have been shown to do so.

A. Neurohumoral Factors Involved in the Ovulation Mechanism

In addition, numerous experiments carried out in recent years have provided evidence that the release of the pituitary gonadotropins necessary for ovulation in the cow, as well as in other "spontaneously" ovulating species, is influenced, and perhaps even regulated, through the hypothalamus by a neurohumoral mechanism.

The experiments with laboratory animals establishing the basis for a neurohumoral concept of ovulation have, for the most part, been done since 1946 and have been summarized by Markee *et al.* (122, 123, 124), Harris (93), and Hansel (88, 90). A series of experiments conducted since 1951 show that a similar mechanism exists in the cow. Hansel and Trimberger (84) found that the administration of the parasympathetic-blocking drug, atropine, to dairy heifers at the beginning of estrus blocked ovulation, while ovulation occurred earlier than the normal time in 4 of 5 heifers given both atropine and chorionic gonadotropin at the beginning of estrus. These results were interpreted as indicating that a neurogenic mechanism having a cholinergic component is involved in the release of the pituitary gonadotropin necessary for ovulation in the cow. It was subsequently found that estradiol given at the beginning of estrus did not hasten the time of ovulation (85), but progesterone given at the beginning of estrus hastened ovulation (87). The latter result, when considered together with the granulosa cell changes observed prior to ovulation and the presence of progesterone in bovine follicular fluid prior to ovulation, suggest that a preovulatory rise in the blood level of progesterone may be an integral part of the ovulatory mechanism in the cow. Fraps (69) has summarized evidence indicating that progesterone causes the release of the ovulation-inducing hormone in the hen and that it does so through a neural mechanism.

Hough *et al.* (103) presented evidence to indicate that progesterone given at the beginning of estrus is incapable of overcoming atropine blockage of ovulation, a result which suggests that the ovulation-hasten-

ing effect of progesterone in normal heifers is due to its influence on the hypothalamus rather than its direct effect on the anterior pituitary. These authors also reported that relatively small doses of epinephrine given at the beginning of estrus did not hasten ovulation.

These results and those of numerous experiments conducted with laboratory animals have given rise to a concept of neurohumoral control of the release of the pituitary gonadotropins necessary for ovulation. The basic features of this concept include: (1) The release of one or more neurohumoral substances by certain hypothalamic nuclei as a result of the stimulus of copulation in species in which ovulation is "induced" and unknown stimuli in "spontaneously" ovulating species; (2) the transport of this chemical mediator(s) to the cells in the anterior pituitary by way of the hypophyseal portal vascular system and the blood sinusoids of the anterior pituitary to cause the release of gonadotropin(s) necessary for ovulation; (3) the existence of a cholinergic and an adrenergic component acting in sequence in the neurohumoral release mechanism.

The striking affinity of the delta cells in the bovine pituitary for the blood sinusoids and the nature and rapidity of the degranulation process which these cells undergo in late proestrus further suggest that this process is controlled by a blood-borne humoral substance. This process, as described by Jubb and McEntee (106), begins in the medulla adjacent to the zona tuberalis and sweeps on a broad front through the whole pars distalis proper. The zone of transition from degranulated cells through those that are active to the cells that are not yet active can sometimes be included in one medium power field of the microscope. It is not yet certain whether the process represents the secretion of FSH or LH or both, but ovulation does not occur in its absence.

B. The Nature of the Neurohumoral Substances

As knowledge of the pituitary-hypothalamic relationships has accumulated, questions concerning the nature of the exteroceptive pathways to the hypothalamus and the stimuli which most effectively activate them in various species, and the nature of the neurohumoral substances acting between the hypothalamus and the anterior pituitary have assumed considerable importance.

Sawyer *et al.* (158) concluded on the basis of considerable evidence (121, 156) that the final humoral agent which activates the pituitary is adrenergic in nature, but other workers have questioned this conclusion. Donovan and Harris (58) reported that epinephrine injected into the pituitary of rabbits did not cause ovulation when the pH of the solution was adjusted to 6.9-7.1, but did cause ovulation in 3 of 15 rab-

bits when the solution was made acid, indicating that the pH of the solution injected influenced gonadotropin release. Intravenous injections of epinephrine have not caused ovulation except in atropinized rabbits, in which relatively enormous doses have been effective (158). Other unphysiological substances, such as copper acetate, have been shown to induce ovulation when injected intravenously (66) or directly into the pituitary (157).

Recent findings (11, 159) indicate that the posterior pituitary hormones, oxytocin and vasopressin, are formed in hypothalamic nuclei, and raise the distinct possibility that these neurohypophyseal hormones or related substances may be concerned in activation of the anterior pituitary. "Neurosecretory material," which stains selectively with chrome-hematoxylin (73), originates in the supraoptic and paraventricular nuclei and migrates down the nerve axons to their endings in the posterior pituitary. These axons come in close approximation to the primary capillary plexus of the hypophyseal portal vessels in the region of the median eminence, and it has been proposed by Benoit and Assenmacher (14) and Scharrer and Scharrer (159) that the chemotransmitter substance responsible for controlling gonadotropic function of the anterior pituitary may be contained in this "neurosecretory material."

All of the evidence relating this Gomori-staining material to the posterior pituitary hormones cannot be reviewed here, but the material stained is apparently a carrier substance for the hormones (197). Adams and Sloper (1) have found that cystine in pituitary and hypothalamic sections has a distribution almost identical to that of the neurosecretory material as described by Bargmann and Scharrer (11). The neurohypophyseal hormones contain cystine, and these results provide further confirmation of the hypothalamic origin of these hormones.

These results suggest the possibility of a close integration of the functions of the anterior and posterior lobe hormones, and there is considerable supportive evidence for such an integration in the cow. Van Demark and Hays (183) showed that sexual stimulation, including sight of the bull, nuzzling of the vulva, mounting, and copulation all cause increased uterine motility. These workers (183) also demonstrated that manual manipulation of the bovine reproductive tract caused an output of oxytocin, as measured by increased intramammary pressure. The observations may be related to those of Marion *et al.* (119), who found that sterile copulation in heifers caused ovulation to occur earlier than in unmated controls, and to the finding of Wilbank and Casida (194) that hysterectomy in the cow causes maintenance of the corpus luteum and delayed returns to estrus.

Although these and many similar experiments carried out with rabbits and rats (78, 107, 149) suggest a relationship between oxytocin release and gonadotropin secretion, none of them offers any proof that the latter are secreted in response to the former.

Gonadotropin secretion associated with stimuli which cause oxytocin secretion might conceivably be due to vasopressin, or even some other substance of hypothalamic origin. Stimuli which evoke the release of oxytocin often cause the concomitant release of vasopressin and vice versa (89).

Shibusawa *et al.* (161, 162, 163) suggested, on the basis of changes in urinary 17-ketosteroid excretion following oxytocin injections, that oxytocin stimulates gonadotropin secretion in the human, but it is not certain whether the ketosteroid fractions measured are of adrenal or gonadal origin (89, 137). Benson and Folley (15) reported that oxytocin injections in lactating rats retard the mammary gland involution that normally follows removal of the suckling young, suggesting that the release of prolactin, or perhaps other anterior pituitary hormones concerned in lactation, can be stimulated by treatment with oxytocin.

These indirect indications that oxytocin of hypothalamic origin might be involved in regulating anterior pituitary gonadotropin secretion led to experiments testing the effects of oxytocin on estrus and ovulation in the cow. In the first of these experiments, Hansel *et al.* (89) found that oxytocin (50 I.U. intravenously plus 50-100 units subcutaneously) administered to heifers at the beginning of estrus significantly hastened the time of ovulation. The oxytocin preparations used were apparently free of gonadotropin, since they produced no increase in ovarian or uterine weight in hypophysectomized rats. In a subsequent experiment, essentially the same doses of oxytocin proved incapable of overcoming the ovulation-blocking effect of atropine when both substances were given at the beginning of estrus. Chorionic gonadotropin is the only substance yet tested which is capable of overcoming atropine blockage of ovulation in the cow.

In the most recent, and perhaps the most significant, experiment in this series, Armstrong and Hansel (3) found that properly timed daily injections of a purified oxytocin preparation,¹ apparently free of gonadotropins, caused major alterations in the bovine estrous cycle. Seven daily injections of oxytocin (50 I.U. intravenously and 100 I.U. subcutaneously), the first of which was given during estrus and prior to ovulation, were followed by a normal estrus and ovulation 2-5 days after the last injection. The corpora lutea formed while the oxytocin injection

¹ Armour's Purified Oxytocic Principle (P.O.P.).

tions were being made appeared to be nonfunctional, or at best only partly functional. The subsequent estrous cycles were of normal length and ovulation occurred at the normal time.

The fact that exogenous oxytocin or some other substance contained in the oxytocin preparations used is able to produce these alterations in the bovine estrous cycle does not necessarily prove that oxytocin is one of the neurohumoral substances of hypothalamic origin involved in the regulation of gonadotropin secretion by the anterior pituitary, but this fact, together with the supporting evidence previously cited, certainly suggests that such is the case. It is not known whether oxytocin injections produce these effects by increasing the secretion of FSH or LH or by inhibiting the secretion of whatever gonadotropin is responsible for maintenance of the corpus luteum in the cow.

The experimental results obtained to date might be explained by assuming that oxytocin increases the output of one of the anterior pituitary gonadotropins and that the release of the other is brought about by a second neurohumoral mechanism having a cholinergic (atropine-blocked) and an adrenergic (dibenamine blocked) component. Cross (54) has shown that two such systems are present in the hypothalamus of the rabbit. One is concerned with the secretion of oxytocin, which follows stimulation of the paraventricular and supraoptic nuclei. The other is concerned with an activation of the sympathetic outflow and the secretion of adrenaline by the adrenal medulla, which results from stimulation of the dorsal, lateral, or posterior areas of the hypothalamus.

Additionally, there is evidence that the oxytocin release mechanism in rats contains both a cholinergic and an adrenergic component (76), and the possibility exists that atropine and dibenamine block ovulation by preventing the release of oxytocin from the hypothalamic nuclei. If only a single mechanism of this sort were involved, however, oxytocin injections should overcome atropine blockage of ovulation, and this proved not to be the case in the cow.

C Present Status of Knowledge Concerning Ovulation in the Cow

It is not possible to give a complete explanation of the mechanism of ovulation in the cow. Apparently, our basic knowledge of the interactions of the ovarian and hypophyseal hormones should be enlarged and revised to include the concept that the secretion of the pituitary gonadotropins necessary to cause follicle development and ovulation is brought about by one or more neurohumoral substances produced in the hypothalamus and carried to the anterior pituitary by the hypophyseal portal vascular system. At present, it seems possible that oxytocin is one

of these neurohumors in the cow. The neurohumoral mechanism for ovulation also appears to contain a cholinergic and an adrenergic component, since ovulation can be blocked in several species by either atropine or dibenamine. The paradoxical effects of progesterone and estradiol on estrus and ovulation when given in various amounts at varying stages of the cycle are probably best explained by assuming that their actions are mediated through the hypothalamus.

REFERENCES

1. Adams, G. W. M., and Sloper, J. C., *J. Endocrinol.* **13**, 221 (1956).
2. Alexander, F., *J. Comp. Pathol. Therap.* **55**, 140 (1945).
3. Armstrong, D. T., and Hansel, W., *Federation Proc.* **17**, Part I, p. 18, abstr. (1958).
4. Aschbacher, P. W., Smith, V. R., and Stone, W. H., *J. Animal Sci.* **15**, 952 (1958).
5. Asdell, S. A., de Alba, J., and Roberts, S. J., *J. Animal Sci.* **4**, 277 (1945).
6. Asdell, S. A., "Patterns of Mammalian Reproduction." Comstock, New York, 1948.
7. Asdell, S. A., de Alba, J., and Roberts, S. J., *Cornell Vet.* **39**, 389 (1949).
8. Asdell, S. A., "Cattle Fertility and Sterility." Little, Brown, Boston, Mass., 1955.
9. Austin, C. R., and Braden, A. W. H., *J. Biol. Sci.* **7**, 179 (1954).
10. Autrup, E., and Rasbech, N. O., *Nord. Veterinarmed.* **3**, 40 (1951).
11. Bargmann, W., and Scharer, E., *Am. Scientist* **39**, 255 (1951).
12. Bearden, H. J., Hansel, W., and Bratton, R. W., *J. Dairy Sci.* **39**, 312 (1956).
13. Bearden, H. J., *J. Dairy Sci.* **40**, 638 (1957) (abstr.).
14. Benoit, J., and Assenmacher, I., *Arch. anat. microscop.* **42**, 334 (1953).
15. Benson, G. K., and Folley, S. J., *J. Endocrinol.* **16**, 189 (1957).
16. Bitman, J., and Sykes, J. F., *Proc. 3rd Symposium Reproduction and Infertility Colo. State Univ. Ft. Collins, Colo.* (1958), in press.
17. Black, W. G., Simon, J., McNutt, S. H., and Casida, L. E., *J. Dairy Sci.* **36**, 588 (1953).
18. Black, W. G., Ulberg, L. C., Christian, R. E., and Casida, L. E., *J. Dairy Sci.* **36**, 274 (1953).
19. Black, W. G., Ulberg, L. C., Kidder, H. E., Simon, J., McNutt, S. H., and Casida, L. E., *Am. J. Vet. Research* **14**, 179 (1953).
20. Black, W. G., Simon, J., McNutt, S. H., and Casida, L. E., *Am. J. Vet. Research* **14**, 318 (1953).
21. Black, W. G., Simon, J., Kidder, H. E., and Wiltbank, J. N., *Am. J. Vet. Research* **15**, 247 (1954).
22. Blair, G. W. S., Folley, S. J., Malpress, F. H., and Coppen, F. M. V., *Biochem. J.* **35**, 1039 (1941).
23. Blair, G. W. S., Folley, S. J., Coppen, F. M. V., and Malpress, F. H., *Nature* **147**, 453 (1941).
24. Blair, G. W. S., and Glover, F. A., *Proc. 3rd Intern. Congr. Animal Reproduction Cambridge Univ., Cambridge, Engl. Sect. I Physiol.* p. 56. (1956).
25. Bone, J. F., *Am. J. Vet. Research* **15**, 542 (1954).
26. Bonfert, A., *Proc. 3rd Intern. Congr. Animal Reproduction Cambridge Univ., Cambridge, Engl. Sect. I Physiol.* p. 77 (1958).

- 27 Bonfert, A, Bulgrin, K D, and Mu, F, *Proc 3rd Intern Congr Animol Reproduction Cambridge Univ Cambridge, Engl Sect I Physiol* p 81 (1956)
- 28 Bourne, G, and Zuckerman, S, *J Endocrinol* 2, 268 (1940)
- 29 Boyland, E, *Biochem J* 40, 334 (1946)
- 30 Brambell, F W R, in "Marshall's Physiology of Reproduction" (A S Parkes, ed), Vol I, Pt 1, p 481, Longmans Green, London, 1956
- 31 Branton, C, Hall, J C, Stone, E J, Lank, R B, and Frye, J B, Jr, *J Dairy Sci* 40, 628 (1957)
- 32 Brock, H, and Rowson, L E, *J Agr Sci* 42, 479 (1952)
- 33 Brody, S, and Frankenbaeh, R F, *Missouri Univ Agr Expt Sto Research Bull No* 349 (1942)
- 34 Buch, N C, Tyler, W J, and Casida, L E, *J Dairy Sci* 38, 73 (1955)
- 35 Carvaglios, R, and Cilotti, R, *J Endocrinol* 15, 273 (1957)
- 36 Casida, L E, Chapman, A B, and Rupel, W, *J Agr Research* 50, 953 (1935)
- 37 Casida, L E, and Venzke, W G, *Proc Am Soc Animal Production* 29, 221 (1936)
- 38 Casida, L E, Nalbandov, A V, McShan W H, Meyer, R K, and Wisnicky, W, *Proc Am Soc Animal Production* 33 302 (1940)
- 39 Casida, L E, Meyer, R K, McShan, W H, and Wisnicky, W, *Am J Vet Research* 4, 76 (1943)
- 40 Casida, L E, in 'The Problem of Fertility' (E T Engle, ed), p 49, Princeton Univ Press, Princeton, New Jersey, 1946
- 41 Casida, L E, and Wisnicky, W, *J Animal Sci* 9, 238 (1950)
- 42 Cesar, J, *Compt rend soc biol* 122, 1237 (1936)
- 43 Chang, M C, *Proc 1st Natl Egg Transfer Conf Foundation Appl Research San Antonio, Texas* (1949)
- 44 Chang, M C, *Noture* 168, 697 (1951)
- 45 Chang, M C, *Noture* 175, 1036 (1955)
- 46 Chang, M C, *Noture* 179, 258 (1957)
- 47 Chapman, A B, and Casida, L E, *Proc Am Soc Animal Production* 27, 57 (1934).
- 48 Chapman, A B, and Casida, L E, *J Agr Research* 54, 417 (1937)
- 49 Christian, R E, and Casida, L E, *J Animal Sci* 7, 546 (1948) (abstr)
- 50 Clapp, H A, *Prac Am Soc Animal Production* 30, 259 (1937)
- 51 Clark, C F, *J Am Vet Med Assoc* 88, 62 (1936)
- 52 Cole, H H, *Am J Anat* 46, 261 (1936)
- 53 Cole, H H, *J Vet Research* 11, 161 (1950)
- 54 Cross, B A, *Proc 3rd Intern Congr Animal Reproduction Cambridge Univ Cambridge, Engl Sect I Physiol* p 72 (1956)
- 55 Cupps, P T, and Asdell, S A, *J Animal Sci* 3, 351 (1941)
- 56 Cupps, P T, Lohen, R C, and Mend, S W, *J Dairy Sci* 39, 155 (1956)
- 57 De Lange, M, *Onderstepoort J Vet Sci Animal Ind* 24 125 (1950)
- 58 Donovan, B T, and Harris, G W, *J Physiol (London)* 132, 577 (1956)
- 59 Dowling D F, *J Agr Sci* 39, 371 (1919)
- 60 Dutt, R H, *Iowa State Coll J Sci* 28, 55 (1933)
- 61 Edgar, D G, *Nature* 170, 513 (1952).
- 62 Edgar, D G, *Biochem J* 54, 50 (1953)
- 63 Edgar, D G, *Nature* 173 639 (1954).
- 64 Edwards J, *Vet Record* 62, 310 (1950).

65. Evans, E. I., and Muller, F. W., *Am. J. Physiol.* **116**, 44 (1936).
66. Fevold, H. L., Hisaw, F. L., and Greep, R. O., *Am. J. Physiol.* **117**, 68 (1936).
67. Foley, R. G., and Greenstein, J. S., *Proc. 3rd Symposium Reproduction and Infertility*, Colo. State Univ. Ft. Collins, Colo. (1958), in press.
68. Folley, S. J., and Malpress, F. H., *Proc. Roy. Soc.* **B132**, 164 (1944).
69. Fraps, R. M., in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 2, p. 661. Butterworths, London, 1955.
70. Garm, O., *Acta Endocrinol.* **2**, Suppl. **3** (1949).
71. Garm, O., and Skjerven, O., *Nord. Veterinarmed.* **4**, 1098 (1952).
72. Glover, F. A., *Nature* **172**, 255 (1953).
73. Comori, G., *Am. J. Pathol.* **17**, 395 (1911).
74. Gorski, J., Erb, R. E., and Brinkman, D. C., *J. Animal Sci.* **16**, 698 (1957).
75. Graham, E. F., and Dracy, A. E., *J. Dairy Sci.* **36**, 772 (1953).
76. Grosvenor, C. E., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* **95**, 719 (1957).
77. Guilbert, H. R., and McDonald, A., *Proc. Am. Soc. Animal Production* **26**, 252 (1933).
78. Hammond, J., "Reproduction in the Rabbit." Oliver and Boyd, Edinburgh, 1925.
79. Hammond, J., "The Physiology of Reproduction in the Cow." Cambridge Univ. Press, London and New York, 1927.
80. Hammond, J., Jr., and Bhattacharya, P., *J. Agr. Sci.* **34**, 1 (1944).
81. Hansel, W., thesis, Cornell Univ., Ithaca, New York (1949).
82. Hansel, W., Asdell, S. A., and Roberts, S. J., *Am. J. Vet. Research* **10**, 221 (1949).
83. Hansel, W., and Asdell, S. A., *J. of Dairy Sci.* **34**, 37 (1951).
84. Hansel, W., and Trimberger, C. W., *J. Animal Sci.* **10**, 719 (1951).
85. Hansel, W., Trimberger, C. W., and Bearden, H. J., *J. Animal Sci.* **11**, 793 (1952) (abstr.).
86. Hansel, W., and Asdell, S. A., *J. Animal Sci.* **11**, 346 (1952).
87. Hansel, W., and Trimberger, C. W., *J. Dairy Sci.* **35**, 65 (1952).
88. Hansel, W., *Iowa State Coll. J. Sci.* **23**, 1 (1953).
89. Hansel, W., Armstrong, D. T., and McEntee, K., *Proc. 3rd Symposium Reproduction and Infertility Colo. State Univ., Ft. Collins, Colo.* (1958), in press.
90. Hansel, W., *Intern. J. Fertility* **3**, 42 (1958).
91. Hansel, W., unpublished observations, 1958.
92. Hansson, A., *Proc. Brit. Soc. Animal Production* p. 51 (1956).
93. Harris, G. W., "Neural Control of the Pituitary Gland." Arnold, London, 1955. *Monographs Physiol. Soc. No. 3*, 298 (1955).
94. Haskins, A. L., *Proc. Soc. Exptl. Biol. Med.* **73**, 439 (1950).
95. Hawk, H. W., Tyler, W. J., and Casida, L. E., *J. Dairy Sci.* **37**, 252 (1954).
96. Hays, R. L., and Van Demark, N. L., *Am. J. Physiol.* **172**, 557 (1953).
97. Hays, R. L., and Van Demark, N. L., *Am. J. Physiol.* **172**, 553 (1953).
98. Herman, H. A., and Edmondson, J. H., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 462* (1950).
99. Herrick, J. B., *Am. J. Vet. Research* **12**, 278 (1951).
100. Hill, D. L., Petersen, W. E., and Cohen, S. H., *J. Dairy Sci.* **37**, 355 (1954).
101. Hofflinger, H., *Acta Anat. Suppl.* **5**, 1 (1947).
102. Hofstad, M. S., *Cornell Vet.* **31**, 379 (1941).
103. Hough, W. H., Bearden, H. J., and Hansel, W., *J. Animal Sci.* **14**, 739 (1955).

- 104 Joubert, D M, *J Agr Sci* 45, 164 (1954)
- 195 Jubb, K V, and McEntee, K, *Cornell Vet* 45, 576 (1955)
- 196 Jubb, K V, and McEntee, K, *Cornell Vet* 45, 593 (1955)
- 107 Krehbiel, R H, and Carstens, H B, *Am J Physiol* 125, 571 (1939)
- 108 Krupski, A, *Schweiz Arch Tierheilk* 59, 1 (1917)
- 109 Kupfer, M, *Denkschr schwed naturforsch Ges* 56, 1 (1920)
- 110 Lammung, G E, and Rowson, L E, *Proc 2nd Intern Congr Physiol and Pathol Animal Reproduct and Artificial Insemination, Copenhagen* 1, 144 (1952)
- 111 Lammung, G E, and Rowson, L E, *Proc Roy Soc Med* 46 (5), 387 (1953)
- 112 Larson, G L, and Bayley, N D, *J Dairy Sci* 38, 549 (1955)
- 113 Lasley, J F, and Bogart, R, *Missouri Univ Agr Expt Sta Research Bull No* 376 (1943)
- 114 Lombard, L, Morgan, B B, and McNutt, S H, *J Morphol* 86, 1 (1959)
- 115 Lutwak-Mann, C, *J Agr Sci* 44, 477 (1954)
- 116 Mandl, A M, Zuckerman, S, and Patterson, H D, *J Endocrinol* 8, 347 (1952)
- 117 Marden, W G R, *Proc 2nd Intern Congr Physiol and Pathol Animal Reproduction and Artificial Insemination, Copenhagen* 1, 154 (1952)
- 118 Marden, W G R, *Endocrinology* 50, 456 (1952)
- 119 Marion, G B, Smith, V R, Wiley, T E, and Barrett, G R, *J Dairy Sci* 33, 885 (1950)
- 120 Marion, G B, and Smith V R, *J Dairy Sci* 35, 71 (1952)
- 121 Markee, J E, Sawyer, C H, and Hollinshead, W H, *Endocrinology* 38, 345 (1946)
- 122 Markee, J E, Sawyer, C H, and Hollinshead, W H, *Recent Progr in Hormone Research* 2, 117 (1948)
- 123 Markee, J E, *Ann Rev Physiol* 13, 367 (1951)
- 124 Markee, J E, Everett, J W, and Sawyer, C H, *Recent Progr in Hormone Research* 7, 139 (1952)
- 125 McEntee, K, *Intern J Fertility* 3, 129 (1958)
- 126 McNutt, G W, *J Am Vet Med Assoc* 65, 558 (1924)
- 127 Melampy, R M, Emmerson, M A, Rakes, J M, Hanka, L J, and Eness, P G, *J Anim Sci* 16, 967 (1957)
- 128 Melton, A A, Berry, R O, and Butler, O D, *J Anim Sci* 10, 993 (1951)
- 129 Meyer, K, *Cold Spring Harbor Symposia Quant Biol* 6, 91 (1938)
- 130 Moeller, A N, and Van Demark, N L, *J Anim Sci* 10, 988 (1951)
- 131 Morrison, F. B, "Feeds and Feeding," 22nd ed Morrison, Ithaca, New York, 1956
- 132 Moss, S, Wrenn, T R, and Sykes, J F, *Anat Record* 120, 409 (1954)
- 133 Moss, S, Wrenn, T R, and Sykes, J F, *Endocrinology* 55, 201 (1954)
- 134 Murphy, H S, *Vet Practit Bull Iowa State Coll Agr* 25, 153 (1920)
- 135 Neller, J E, and Cole, H H, *J Animal Sci* 15, 650 (1956)
- 136 Neller, J E, and Cole, H H, *J Animal Sci* 16, 151 (1957)
- 137 Nishikawa, M, Olino, F, Ibayashi, H, Ishibashi, C, Motobashi, K, and Watanabe, R, *Endocrinol Japon* 2, 271 (1955).
- 138 See Ito, R H, p 5, in *Northeast Regional Publication No 32, Cornell Univ Agr Expt Sta Bull No* 924 (1957)
- 139 Olds, D, and Seath, D M, *J Dairy Sci* 34, 620 (1951)
- 140 Olds, D, and Seath, D M, *J Animal Sci* 12, 10 (1953).

141. Olds, D., and Van Demark, N. L., *Am. J. Vet. Research* 18, 587 (1957).
142. Olds, D., and Van Demark, N. L., *Fertility and Sterility* 8, 345 (1957).
143. Papanicolaou, G. N., Traut, H. F., and Marchetti, A. A., "The Epithelia of Woman's Reproductive Organs." Commonwealth Fund, New York, 1948.
144. Perkins, J. R., Olds, D., and Seath, D. M., *J. Dairy Sci.* 37, 1158 (1954).
145. Pou, J. W., Henderson, C. R., Asdell, S. A., Sykes, J. F., and Jones, R. G., *J. Dairy Sci.* 36, 909 (1953).
146. Raeside, J. I., and Turner, C. W., *J. Dairy Sci.* 38, 1334 (1955).
147. Reece, R. P., and Turner, C. W., *J. Dairy Sci.* 21, 37 (1938).
148. Reineke, E. P., and Soliman, F. A., *Iowa State Coll. J. Sci.* 28, 67 (1953).
149. Reynolds, S. R. M., *Am. J. Physiol.* 92, 420 (1930).
150. Roark, D. B., and Herman, H. A., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 455* (1950).
151. Roberts, S. J., "Veterinary Obstetrics and Genital Diseases." S. J. Roberts, Ithaca, New York. Distributed by Edward Bros., Ann Arbor, Michigan, 1956.
152. Rottensten, K., and Touchberry, R. W., *J. Dairy Sci.* 40, 1457 (1957).
153. Rowson, L. E., *J. Endocrinol.* 7, 260 (1951).
154. Rowson, L. E., Lamming, G. E., and Fry, R. M., *Vet. Record* 65, 335 (1953).
155. Rowson, L. E., Lamming, G. E., and Fry, R. M., *Nature* 171, 749 (1953).
156. Sawyer, C. H., Everett, J. W., and Markee, J. E., *Endocrinology* 44, 218 (1949).
157. Sawyer, C. H., and Markee, J. E., *Endocrinology* 46, 177 (1950).
158. Sawyer, C. H., Markee, J. E., and Everett, J. W., *Endocrinology* 46, 536 (1950).
159. Scharrer, E., and Scharrer, B., *Recent Progr. in Hormone Research* 10, 183 (1954).
160. Shannon, E. P., Salisbury, G. W., and Van Demark, N. L., *J. Animal Sci.* 11, 355 (1952).
161. Shibusawa, K., Saito, S., Fukuda, M., Koibuchi, E., Kawai, T., and Yamamoto, T., *Endocrinol. Japon.* 2, 313 (1955).
162. Shibusawa, K., Saito, S., Fukuda, M., Kawai, T., Yamada, H., and Tomizawa, K., *Endocrinol. Japon.* 2, 189 (1955).
163. Shibusawa, K., Saito, S., Fukuda, M., Kawai, T., Yamada, H., and Tomizawa, K., *Endocrinol. Japon.* 2, 181 (1955).
164. Sisson, S., and Crossman, J. D., "The Anatomy of Domestic Animals," 4th ed. Saunders, Philadelphia, Pennsylvania, 1953.
165. Skjerven, O., *Acta Endocrinol.* 22, Suppl. 25 (1956).
166. Smith, V. R., McShan, W. H., and Casida, L. E., *J. Dairy Sci.* 40, 443 (1957).
167. Sorensen, A. M., Hansel, W., Hough, W. H., Armstrong, D. T., McEntee, K., and Bratton, R. W., *Cornell Univ. Expt. Sta. Research Bull.* 936, in press.
168. Spielman, A. A., Petersen, W. E., Fitch, J. B., and Pomeroy, B. S., *J. Dairy Sci.* 28, 329 (1945).
169. Sykes, J. F., Moss, S., and Wrenn, T. R., *Proc. Centennial Symposium Reproduction and Infertility Mich. State Univ., East Lansing, Mich.* p. 63 (1955).
170. Szego, C. M., and Roberts, S., *Proc. Soc. Exptl. Biol. Med.* 61, 161 (1946).
171. Trimberger, C. W., *J. Dairy Sci.* 24, 819 (1941).
172. Trimberger, C. W., *Nebraska Univ. Agr. Expt. Sta. Research Bull. No. 153* (1948).

- 173 Trimberger, C W, *J Dairy Sci* 37, 1042 (1954)
- 174 Trimberger, C W, and Hansel, W, *J Animal Sci* 14, 224 (1955)
- 175 Trimberger, C W, *J Dairy Sci* 39, 448 (1956)
- 176 Turner, C W, Frank, A H, Lamas, C H, and Nibler, C W, *Missouri Univ Agr Expt Sta Bull No* 150 (1930)
- 177 Ulberg, L C, Christian, R E, and Casida, L E, *J Animal Sci* 10, 752 (1951)
- 178 Ulberg, L C, Boulware, R, Deese, R, and Lindley, C E, *J Animal Sci* 13, 1033 (1954) (abstr)
- 179 Ulberg, L C, *Prac Centennial Symposium Reproduction and Infertility Mich State Univ, East Lansing, Mich* p 104 (1955)
- 180 Umbrough, R E, *Am J Vet Research* 10, 295 (1949)
- 181 Van Demark, N L, and Salisbury, C W, *J Animal Sci* 9, 307 (1950)
- 182 Van Demark, N L, and Moeller, A N, *Am J Physiol* 165, 674 (1951)
- 183 Van Demark, N L, and Hays, R L, *Iowa State Coll J Sci* 28, 107 (1953)
- 184 Vandeplasse, M, and Paredis, F, *Nature* 162, 813 (1948)
- 185 Warnick, A C, *J Animal Sci* 14, 1003 (1955)
- 186 Weber, A F, Morgan, B B, and McNutt, S H, *Am J Anat* 83, 309 (1948)
- 187 Weeth, H J, and Herman, H A, *Missouri Univ Agr Expt Sta Research Bull No* 501 (1952)
- 188 Willett, E L, *J Dairy Sci* 33, 381 (1950)
- 189 Willett, E L, McShan, W H, and Meyer, R K, *Proc Soc Exptl Biol Med* 79, 396 (1952)
- 190 Willett, E L, *Iowa State Coll J Sci* 28, 83 (1953)
- 191 Willett, E L, Buckner, P J, and Larson, G L, *J Dairy Sci* 36, 520 (1953)
- 192 Willett, E L, Buckner, P J, and McShan, W H, *J Dairy Sci* 36, 1083 (1953)
- 193 Willett, E L, *J Dairy Sci* 39, 695 (1956)
- 194 Wiltbank, J N, and Casida, L E, *J Animal Sci* 15, 134 (1956)
- 195 Wiltbank, J N, Cook, A C, Davis, R E, and Warwick, E J, *J Animal Sci* 16, 1100 (1957) (abstr)
- 196 Woods, M C, thesis, Cornell Univ, Ithaca, New York, 1950
- 197 Zuckerman, S, *Lancet* 266, 739 (1954)
- 198 Zupp, B A, *Vet Practit Bull Iowa State Coll Agr* 25 123 (1926)

CHAPTER 8

The Estrous Cycle of the Mare

VICTOR R BERLINER

	<i>Page</i>
I The Breeding Season of Mares	267
II The Pattern of the Estrous Cycle of the Mare ✓	271
A The <u>Duration</u> of the Estrous Cycle	271
B The Duration of <u>Estrus</u>	272
✓ C The Diestrous Period	274
D Foal Heat and the Interval between Foaling and First Estrus	276
III Physiological and Histological Changes in the Reproductive System	277
A Ovarian Changes and Ovulation	277
B Changes in the Cervical Tract	281
C Histological Changes in the Reproductive Tract	282
✓ D pH of Vaginal Secretions	284
IV The Behavioral Pattern of the Cyclic Mare	284
V Adaptation of the Breeding Program to Cyclic Events	285
References	287

I THE BREEDING SEASON OF MARES

The information in the literature pertaining to the mare as a seasonal breeder is confusing and inconsistent. Dukes (33) states that the "breeding season is usually in the spring, from March to July, but, if she is not bred at this time, she may continue to experience estrous cycles for a variable period depending on the individual." According to Rice (72), "mares are polyestrous for part of the year, generally from March through July and sometimes on into the fall months." Asdell (4) states that "seasonal cycles begin about March and usually continue, in the unbred mare, into August, but many breed in the fall and winter in England."

Heape, as early as 1900, made the frequently quoted statement that the "mare is a polyestrous animal with a tendency toward monestrum" (35). Since the wild species of the equine family are reported to be monestrous (35), the "tendency toward monestrum" in the modern mare could be interpreted as an atavistic trait, and the polyestrous property must be an acquired character brought on by breed improvement and domestication. Lagerlof (55) suggested that the modern mare represents a transitional stage from the monestrous to the polyestrous type. The question arises, how far the development toward the polyestrous character has progressed, and how firmly is it established in the modern horse?

Since foals are born in every month of the year, some investigators are inclined to consider the mare as polyestrous capable of reproducing

without seasonal restrictions (2, 37, 38, 76). According to others, the mare achieves a high level of reproductive efficiency only during a more restricted season of the year, and breeding efficiency is low "at the start of the breeding season" and improves as the season advances (5, 7, 23, 35, 41, 43, 45, 57).

The main confusion seems to arise from a misinterpretation of the terms "polyestrous" and "breeding season." It is assumed that if, and when, the mare shows sexual activity by exhibiting mating desire for the first time in the season, she is also automatically in a state of being able to reproduce. Some mares, but not entire breeds, are endowed with this property and can produce offspring at any season of the year. They are truly polyestrous. But for the majority of the mare population, this apparently does not hold true. Even though they may appear to be polyestrous when judged by their sexual behavior, going through heat cycles throughout most or all of the year, reproduction takes place in a more restricted season. The main trouble seems to concern principally the period referred to as "the early part of the breeding season." It is recognized that in this period heat cycles are irregular, ovulation often does not take place (30, 37), and fertility is extremely low. Yet, it still is considered as part of the "breeding season." It would be more realistic to consider this period rather as a transitory phase from the season of sexual inactivity to that of full sexual activity, when complete reproductive performance begins to set in. Such transitory or preparatory phase occurs regularly in other species with a restricted breeding season, such as sheep, only here it happens in a manner causing less confusion to the breeder. In the ewe, the first heat period announces the onset of the breeding season; that is, the ewe is not only receptive to the ram, but also capable of conceiving. Preceding the first heat period, there occurs at least one period of "silent heat," during which follicular growth and ovulation take place unaccompanied by sexual receptivity (4, 5, 6, 43, 45). In the mare, just the opposite happens. Sexual receptivity usually precedes the state of complete preparedness of the reproductive system. Thus, the appearance of the first heat period does not always coincide with, and is not necessarily an indicator of, the onset of the true breeding season.

As an example of this type of seasonal periodicity in level of reproductive capacity, the observations made by Satoh and Hoshi (74) on native, semiwild pony mares of Korea can be cited. Even though this study was made on a primitive breed, it apparently is applicable to mares of modern breeding. According to these workers, the breeding season appears to be much longer than it is in reality. After complete sexual and ovarian quiescence typical for this breed, lasting from October to

March, sexual receptivity is exhibited at regular intervals from March until September. However, the period of full reproductive activity, during which estrus occurs regularly and the ovary undergoes complete functional changes terminating in ovulation, exists only during May and June. In the intervening periods, from onset of first sexual manifestations until onset of full ovarian activity, there occurs a transitional stage characterized by incomplete ovarian function, when the growth of follicles does not terminate in ovulation, and when, because of this failure of ovulation, the heat periods tend to be long. To such heat periods they applied the term "pseudoestrus." A period characterized by the occurrence of pseudoestrus sets in again after the true breeding season, starting in July, with regressive changes into an anestrus condition around October. Thus, from the standpoint of estrous behavior alone, these ponies can very well be classified as polyestrous, but from the standpoint of reproductive performance, they are almost monestrous.

When mares of this breed are subjected to domestication, the true breeding season becomes longer and extends from May to July, anestrus is shortened with pseudoestrus continuing well into November, instead of terminating in anestrus in October.

In modern breeds of horses, domestication did not eliminate entirely the season of pseudoestrus, but anestrus has been shortened. Deep anestrus with complete ovarian inactivity is a fairly rare occurrence in modern breeds, instead, the shallow type is more common (18, 19). In shallow anestrus, the ovaries do not regress into complete inactivity, but retain some degree of follicular growth, whereas, in deep anestrus, the ovaries are inactive, shrunken, and hard, with occasional small and apparently nonfunctional follicles. Some mares do not experience anestrus at all, but have a period of subestrus when irregular, low cyclical activity continues. This is expressed by prolonged and often incessant periods of intense mating desire, or by periods of silent heat.

Cashek (23), in a study of sexual cycles of over 1200 Thoroughbred mares, cites the instance where, during early spring, 38 mares stayed in heat for 1562 days out of a possible 2130 from February 14 to April 10. Thus, they were in estrus 71% of this period. Later in the season, the same mares resumed normal rhythmical behavior, and 37 of them became pregnant. This demonstrates that the behavior of a mare during the season of pseudoestrus should not be taken as an indicator for her potential breeding performance during the sexual season.

In studies conducted at the Mississippi Experiment Station (7, 9), mares came into heat the year round, but in the early spring a large number behaved similarly to those described by Cashek. Their fertility in this period was low, even though bred frequently throughout these

long estrous periods. During March and April, conception resulted in only 15% of the heat periods in which the mares were mated, whereas in May, June, and July, the conception rose to 45, 41, and 50%, respectively. Fertility remained high well into December.

Observations made in Europe (1, 20, 21, 30, 53) also show that in the early part of the breeding season, i.e., February to May, breeding efficiency tends to be significantly lower than from May to July. The same trend holds true in the Southern Hemisphere for the corresponding seasons. Quinlan *et al.* (71) working at Onderstepoort, South Africa, observed that estrous cycles continued throughout the year in the majority of mares, but best breeding results were obtained in November (spring), when 56% of the mares became pregnant. No mares settled from April to July, when the greatest irregularities in estrous behavior were common. Some mares went into true anestrus.

Nishikawa *et al.* (69) found the breeding season of the she-ass, in Japan, to be between April and September and the period of sexual rest in December and January. The intervening months are phases of transition to and from the period of sexual activity. In South Africa, the breeding season for donkeys is from October to April (54), but jennets in Mississippi appear to be polyestrous with seasonal variations. In early spring, their pseudoestrous periods are highly irregular and tend to be long. During this time, conception rates are just as unsatisfactory as in mares. During the summer, their breeding efficiency improves and is maintained well into the fall (8, 14).

Mechanisms for controlling reproductive function have been the object of speculation for a long time. Livestock breeders have credited the appearance of green grass, sunshine, and warm weather for initiating sexual activity, but, since sexual activity sets in before these factors make their appearance, in the mare the mechanism must be different. It was, therefore, of great interest when Burkhardt, in England (18, 20), succeeded in demonstrating that in the mare initial stimulation of pituitary activity, and the corresponding activation of ovarian functions is brought on by the increase in daylight in the spring. Nishikawa *et al.* (67) have studied the influence of light in Korean pony mares. The influence of environmental factors on reproduction is considered in Vol. II, Chapters 6 and 7.¹

Probably the most fundamental reason for the peculiar sexual pattern of equines, not only in regard to seasonal periodicity, but also for the cyclic phenomena, is founded in a peculiar arrangement of physiological nature in the mare's pituitary. By way of explanation for this, Hammond

¹ Several investigators have studied the influence of seasonal factors upon gestation length in the mare (15, 49, 51, 52, 60, 70, 73, 76, 77, 78).

(43, 45) suggested a basic principle for the mare: the pituitary of the mare is particularly rich in FSH, but low in LH. As a result of this unbalanced production of gonadotropins, the mare is prone to have long heat periods because at times insufficient LH is produced to make the mature follicle ovulate. During the nonbreeding season, the activity of the pituitary gland as a whole is depressed, varying in degree from individual to individual, resulting in different degrees of ovarian activity from anestrus to subestrus. Later in the season, when the activity of the pituitary rises, FSH still predominates over LH and, only when sufficient LH is available, does ovulation occur, and the heat periods become shorter. As a further result of this peculiar balance in the pituitary, the balance in the ovary is also on the follicular side, while the secretion of progesterone is prone to be deficient (41). However, the situation is apparently more complicated than this. In mares with irregular cycles, unusually high amounts of gonadotropin are circulating in the blood, according to Cole (24), which may indicate that the fault does not lie with the pituitary, but rather with the ovary, which at this stage is not capable of responding properly to gonadotropin. Burkhardt (19) had found that the ovary of an anestrus mare does not respond to extraneous gonadotropin at all, especially if anestrus is deep, but that ovarian stimulation can be induced by first treating the ovary with small amounts of estrogens. Under proper estrogen therapy, the ovary in shallow anestrus responds even to estrogen alone with complete follicular development and ovulation, and the stimulated follicles contain fertile ova (13, 19).

II. THE PATTERN OF THE ESTROUS CYCLE OF THE MARE

The most distinguishing feature of the estrous cycle of the mare and other equines is the relatively long portion occupied by the period of estrus. Whereas in other species, estrus is short enough to be measured in hours, in the mare it lasts for days. The physiological reason for this peculiarity probably lies in the already mentioned characteristic predominance of FSH production in the mare's pituitary with the correspondingly high estrogen balance of ovarian activity, and the low LH-progesterone system on the other side.

A. *The Duration of the Estrous Cycle*

According to Asdell (4), the average length of the estrous cycle is 22 days, with a mean from 19 to 23 days. A tabulation by Andrews and McKenzie (3) of data reported by investigators from many parts of the world shows a range of 7 to 124 days. The extremely long cycles are obviously abnormal, and can be attributed to inclusion of cycles occurring in the prebreeding season, and to long cycles brought on by skipped

heat periods. McKenzie (62) reported that 24% of a mare population he investigated underwent one or more skipped heat periods during a breeding season.

Andrews and McKenzie's data (3) show a comparatively wide range in cycle lengths occurring even during the normal breeding season between April and July. In light mares (grade Thoroughbreds), 63% fell between 17 and 24 days, but long cycles of 29 days made up 10%. Among the draft mares, the modal length was essentially the same, but a fair number showed a peculiar tendency to have very short cycles of 10 to 16 days.

Constantinescu and Mauch (26), investigating 1506 cycles of mares of four "warm blood" breeds, obtained a similar frequency distribution: between 19 and 24 days—56%; 9 and 18 days—19%; and 25 to 33 days—25%. They, too, pointed out that the extremely long cycles are due to missed intervening cycles that made up 36% of all investigated cycles.

Another way of determining the duration of the cycle is that of establishing the interval elapsing between ovulations in successive heat periods. This method should eliminate any errors introduced by wrong interpretations of the psychic behavior of the mare. Yet, Andrews and McKenzie, on applying this procedure, found that the duration of the interovulatory interval, too, varies over a wide range with considerable deviation from the mean, from 12 to 58 days in light mares, and 15 to 41 days in draft mares. The frequency distribution figures obtained in this manner come quite close to those obtained by observation of the mares' behavior. The means for the lengths obtained by both methods are essentially the same, 20.6 and 20.7 days. Long intervals appear here again, so they must be due to skipped periods and anovulatory cycles, since they are multiples of the average values. The peculiarly short intervals of 10 to 16 days are again represented in this series, and therefore must occur.

Similar values for the interovulatory period were also obtained by Cummings (27) with an average of 22 days and a range of 12 to 29 days and by Hancock (47), namely, an average of 21 days, with a range from 15 to 24 days. So, by which ever way one measures the cycle length, an average of 21 to 22 days is obtained.

B. The Duration of Estrus

The estrous period is very variable in length and, therefore, is the main contributing factor for the variation in length of the entire cycle. It is about twice as variable as the interval between heat periods, according to Cummings (27). In his data the average for estrus was 5 days

with a coefficient of variation of 42.8 whereas for diestrus, with an average of 15.9 days, the coefficient of variation was 24.47

Asdell (4) concluded that the average ranges between 4 and 9 days, but added "that a great deal depends upon the method of testing for heat and on the statistical analysis of data, since the mare is apt to go out of heat for a short period and to come in again during what is evidently one full heat period." This latter phenomenon is called "split estrus" and will be discussed later. Published data reviewed by Andrews and McKenzie (3) show a wider range for estrus, 1 to 103 days. It is questionable if such long and obviously irregular periods should be included. Their own data show a range from 1 to 37 days, with a mean of 5.3 days, 74% ranged between 2 and 8 days. Light mares were inclined to have short periods, 40% of the periods were 3 days or less in length. In draft mares, the modal length was 5 days, but the frequency distribution curves, although showing a peak at 5 days, reveal longer estrous periods (up to 11 days) occurring frequently enough to be of practical concern to the breeder. Constantinescu and Mauch (26) found that 73% of 3837 estrous periods were 1 to 5 days long, and only 7% were longer than 8 days. Trum (75) in a study of over 1500 cycles in Thoroughbred mares at the U. S. Army Remount Depot, Fort Robinson, Nebraska, found that 61% of the heat periods were 4 to 6 days long, 11%, 2 and 3 days, 28%, 7 to 9 days, and only 5% over 10 days.

A seasonal variation in the duration of estrus is also noticeable. Trum (75) reported a definite trend toward short heat periods in Thoroughbred mares from spring to midsummer. Periods of less than 4 days' duration made up 44% of the periods in March, but 78% in July. Abnormal periods of over 10 days' duration made up 18% in March, dropped to 7% in April, to 2% in May, and did not occur at all in June and July.

A correlation between the seasonal variation in duration of estrus and fertility seems to exist (7, 9). In Mississippi the month of March is not favorable for the breeding performance, since 34% of the estrous periods lasted longer than 18 days, that is, the mares were in almost continuous estrus. Even though 66% of the periods were of normal duration, 5 to 8 days, the fertility in this part of the season was low. Only 14% of the serviced heat periods resulted in pregnancies. The picture improved in April in regard to the estrous periods, with the number of very long heat periods dropping to 10%. Fertility was still very low, however. In the next 3 months, May through July, the percentage of fertile estrous periods reached a normal level, 41 to 50%, at the same time when the duration of estrus shifted significantly into the short range. In May 65%, and in July 94% of estrous periods were 1 to 6 days in length. A few instances of estrous periods over 10 days long were present in

May, but none in June and July. With the advent of very hot and dry weather, the heat periods showed a tendency to be longer but still in the normal range. The fertility dropped off, then returned to a higher level with the onset of cool fall weather.

The seasonal influences on estrus in jennets in Mississippi is not quite as clear-cut as in mares. Estrous periods of very short duration (1 to 2 days) occur not in the summer but in the fall.

C. The Diestrous Period

Fairly general agreement seems to exist among investigators as to the variability in length of the estrous cycle and estrus in the mare, but the views in regard to the duration of the diestrous period are rather conflicting. This is of practical as well as academic interest because the return of the next heat period is often estimated from the last day of the preceding heat period.

Hammond (40, 45) states that in most cases there is an interval of 16 days between the end of one estrus and the beginning of the successive one because the length of life of the corpus luteum of 15 to 17 days is relatively constant in the mare. Laing (57) expresses a similar viewpoint.

In view of the previously mentioned low activity of the mare's pituitary in LH, it is difficult to visualize how and why that phase of the cycle that is under the regulation of the LH-corpora luteum system should be more stable than the other cyclic phases, unless, by way of speculation, one could assume that it is luteotropin, not LH, that is present in adequate quantities to maintain constancy of the active corpora luteum and diestrus. However, the horse pituitary is said to be low in prolactin (luteotropin) (35). Investigations by others show, on the contrary, that the diestrous period is quite variable. Although Cummings (27) found, by statistical analysis, the diestrous period to be half as variable as the estrous period, his own data show a range from 9 to 22 days. Others, cited by Andrews and McKenzie, report a range between 4 and 83 days, with a mean between 12 and 23 days. Andrews and McKenzie reported finding an average duration of 15.3 days with a range of 5 to 33 days. Again, extremely long diestrus could be due to skipped or unobserved preceding heat periods. The frequency distribution offered by them shows that 73% of diestrous periods fall between 14 and 19 days, but only approximately 35% on day 16, which militates against the strict persistence of a 16-day period. This is further supported by data reported by Trum (75). Maiden mares are inclined to have short diestrous periods; in 50% of the cases studied diestrus was shorter than 13 days. Trum makes the very poignant deduction that "this varia-

tion in the interval between estrous periods makes the common practice of teasing mares only on specific days after the last service a precarious practice. It is possible to miss 50% of the mares by teasing on the fourteenth and twenty-first day after the last service as practiced in many studs. There was no correlation between the length of estrus and the length of subsequent interestrus."

Just as unreliable is the practice of checking only on the 16th day or 18th day after the last service or after the mare has gone out of heat, as mares may run every possible combination of long and short heat periods with short or long rest periods (7, 8, 9).

Extremely long intervals between estrous periods extending over the multiples of regular estrous cycles have been reported to occur in mares nursing foals. The problem of foal heat will be taken up in the next section.

As a rule the great majority of foaling mares will resume regular cyclic behavior. Trum's data (75) show that foaling mares have normal diestrous periods more regularly than barren cyclic mares. Data by Cummings (27) show that the first diestrous period following foal heat is practically the same as in cyclic nonfoaling mares, namely, 8 to 28 days, with an average of 14.7 days for the foaling, and 9 to 28 days, with a 15.9 days average, in the nonfoaling mare. Britton and Howell (17), however, noted cases when mares that failed to settle to service given during regular foal heat went into prolonged diestrus lasting an average of 5 months. The length of diestrus was correlated to, or rather governed by, the milk-producing capacity of the mare. Heavy milkers stayed in diestrus up to 6.5 months, and poor milkers for only 2.5 months. This would appear to support the contention of horsebreeders who claim that breeding at foal heat is essential because, according to their theory, this first postpartum estrus is followed by prolonged diestrus. Fortunately, this view is contrary to fact. Even in the series reported above, the incidence of prolonged diestrus is extremely low. In 16 years, in a herd of 36 mares, only 15 mares acted in this manner a total of 23 times.

More frequent are the cases where a nursing mare fails to show estrus because of overanxiousness concerning her foal. Careful testing for estrus with the stallion and checking the condition of the reproductive organs by palpation easily eliminate many such cases from the list of nonbreeders (61). However, lactation interferes with ovarian activity through inhibition of gonadotropin secretion in certain species, such as the sow and rat. It is occasionally reported to occur in the mare. For instance, on the Shetland Islands, pony mares living under poor nutritional conditions rarely produce a foal more than once in two years.

whereas mares of the same breed living under better nutritional conditions will foal yearly (45).

In this instance, it would be more correct to consider the nonbreeding condition as lactation-induced anestrus rather than diestrus.

D. Foal Heat and the Interval between Foaling and First Estrus

Foal heat is the first estrus shortly after parturition. This phenomenon is not restricted to the equine species. It also occurs in a few other species, such as the sow and the camel. The sow is infertile during this heat period, however, because ovulation does not occur. In the mare, foal heat is complete, that is, accompanied by ovulation. It is widely accepted that: foal heat is the most opportune time to get a mare in foal; foal heat occurs persistently on the 9th day after foaling; a mare must be bred on this day because foal heat is followed by anestrus; and, even if a mare is not in heat on the 9th day, she should be force bred and would conceive anyway. Investigations during the last decades have disproved or corrected many of these concepts.

It is recognized now that foal heat, although a regular occurrence in practically all foaling mares, is subject to the typical variability both in regard to time of onset and to duration as any other reproductive phenomenon of the mare. In fact, Cummings (27) stated that, according to his statistical analysis, "more variation is shown in all phases of foaling estrus than appeared in subsequent estrual periods."

The range of the interval between foaling and first estrus may be very wide. Constantinescu and Mauch (26) are of the opinion that estrous periods starting later than 20 to 40 days after foaling should be considered regular cyclic estrous periods, and that the long intervals are caused by skipped or unnoticed foal heats. Among more than 1400 foal heats, they noted only 5% estrous periods of this type; 95% of the foal heats occurred 4 to 17 days after foaling. An interval of 5 to 15 days appears to be quite common (17, 26), but Andrews and McKenzie reported only 36% of the intervals falling between 8 and 12 days, with a mean of 11 days. In 75% of the cases, it began within 14 days after foaling. Trum (75) reported 93% of the mares coming into heat 5 to 15 days after foaling, with 20% on the 8th day, and 33% on the 9th day. He states: "Between the 7th and 10th day after foaling, 77% of mares were in heat. Seventy per cent of the mares, including those that had shown estrus earlier, were in heat on the 9th day following foaling. It is not possible to find so many mares willing to accept the stallion on any other specific day during the estrous cycle." Even though this would speak strongly in favor of breeding during foal heat and even lend support to the practice of "9th day breeding," actual breeding results do

not substantiate this deduction. In Trum's study, 56 healthy mares were bred during foal heat, but only 24 (43%) conceived. The 32 mares that did not conceive at foal heat were rebred on the following heat, and 24 (75%) became pregnant. Furthermore, only 75% of the mares that became pregnant during foal heat produced viable foals. Results emphasizing the risks involved with breeding at foal heat were also reported by Jennings (50): an abortion rate 4 times as great as in mares bred during regular heat, 15% cases of dystocia, 6 times as many dead-born and nonviable foals. The detrimental effects of breeding at foal heat had been recognized, described, and publicized by Williams (79) as early as 1926. Yet, it has persisted and is still practiced. The failures are presumably due to introduction of infection in the uterus by mating. This indicates that the defense mechanism in the uterus is defective at this time. This assumption is strengthened by the finding of Andrews and McKenzie (3) that at foal heat the uterine epithelium is frequently incompletely restored; thus, infections could easily become established (16, 79).

Foal heat itself is reported to last from 1 to 13 days (3), 2 to 13 days (1), 1 to 8 days (4, 7, 21, 59, 63); i.e., is much like cyclic estrous periods. It has the same seasonal variations, shorter in summer and longer in winter and early spring.

Concerning foal heat, the jennet does not differ from the mare (12, 14, 68, 69).

III. PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN THE REPRODUCTIVE SYSTEM

A. Ovarian Changes and Ovulation

The complexity of the cyclic events in equines is caused, not only by peculiarities of endocrine nature, but partially also by the peculiar anatomy and morphology of the ovary. The reproductive organs have been described by Hammond and Wodziaki (46). The reader is also referred to Küpfer's work (54).

The ovary of equines is a bean- or kidney-shaped organ, and larger than in other domestic animals. With sexual maturity, it is approximately 5 to 8 cm. long and 2 to 4 cm. thick. The dimensions vary greatly during the estrous cycle as maturing follicles contribute considerably to its size. At times, they make up the major portion of the ovarian structure. The ovary of the jennet is considerably larger than that of the mare, not infrequently reaching the size of a fist (personal observation).

The distinguishing anatomical feature of the ovary of the sexually mature equine, both the mare and jennet, is the ovulation fossa toward

which the Graafian follicles migrate during the process of maturation and into which they rupture during ovulation. A serous coat covering the mature ovary except at the ovulation fossa prevents ovulation at the surface. The follicles, however, are distributed throughout the ovary; as they increase in size, they at first protrude from the surface and are palpable there, then, during the final stages of maturation, they develop a wedgelike extension toward the ovulation fossa. In young mares and donkeys, however, ovulation is reported to occur on the ovarian surface, as in other species (4, 34, 46, 54).

Determinations of the cyclic phenomena by manual palpation are possible but sometimes difficult, and subject to wrong interpretation. For instance, during one heat period, several follicles may reach considerable size, and the ones not going through ovulation regress rather slowly and remain palpable for longer than the cycle in which they started. Fresh corpora lutea contain fluid and blood-filled cavities and so feel like follicles until luteal tissue develops, when they can be distinguished from follicles by their roughness (2). Also, with advancing age, especially in irregular breeders, the ovary contains more connective tissue and remnants of old corpora lutea, making it unpliant. The cyclic morphological changes, therefore, become less distinguishable by palpation.

During deep anestrus, the quiescent ovaries are small and shrunken, and their surface may be smooth or contain firm nodules of stromal tissue. Occasionally, follicles up to 1 cm. in diameter may be palpated. In shallow anestrus, and when the breeding season approaches, the ovaries have a spongy consistency due to an increased blood supply to the stroma, and follicular activity is demonstrable through the presence of follicles up to 2.5 cm. in diameter (18, 19, 20). In the early spring, these follicles may be sufficiently active as endocrine glands to bring the mare in heat. Even in the normally cyclic mare, follicles of that size or smaller are frequently encountered. Thus, the size of a follicle is neither an indicator of activity nor of maturity.

During the transitional period, before the onset of the true breeding season, mares are inclined to stay in heat for a long time, because at this stage the follicles, after reaching a certain stage of development, fail to ovulate, and continue to produce estrogen in sufficient amount to keep the mare in heat (30, 59). In general, these early follicles regress, but occasionally one may ovulate. The ova are occasionally fertile, since mares bred during such long heat periods may conceive if bred sufficiently often (43, 45, 59). The fault for nonovulation or delay of ovulation apparently rests with the pituitary, which fails to release sufficient quantities of LH to cause ovulation. If LH lack is supplemented by an

extraneous source of gonadotropin, or if the mare's pituitary is stimulated into increased LH production by administration of an estrogen, ovulation can be induced and the estrous period shortened (18, 19, 20, 28, 45, 62, 65, 66). Another peculiarity of the mare is that during the prebreeding season the nonovulating follicles rarely develop into large cysts, as one would expect, because of the one-sided FSH balance. Instead, they regress. According to Burkhardt (20), large cysts are found occasionally after the breeding season, but these, too, regress. Failure to ovulate and regression of the most advanced follicle occurs also during "split-estrus." In this instance, the mare is in heat for several days, out for a few days, and then back in heat. In such instances, the follicle next in line takes over and proceeds toward ovulation (66). Fertility in split-estrus is normal if breeding is performed during the growth of the new follicle (3).

During the normal breeding season, the onset of estrous behavior is associated with the appearance of a palpable follicle that may protrude anywhere on the surface of the ovary but more frequently at the poles. There is no uniformity to the size of the active follicle. Follicles as small as 1 cm. in diameter as well as large ones with a diameter of 7 cm. can be detected at the time of onset of estrus (66). In early heat, most follicles, regardless of size, are tense and their walls taut. As estrus advances, follicular size increases, but the definitive follicle size is not constant at ovulation. A follicle no larger than 2 cm. in diameter may ovulate, as may one the size of a tennis ball (3, 75). The physical condition of the follicle serves as neither a reliable indicator of its maturity nor as a diagnostic means for predicting the time of ovulation. Some investigators claim that the capsule of the follicle becomes softer with impending ovulation, but tenseness may persist or even increase just before ovulation. The migration of the maturing follicle toward the fossa sometimes can be followed by palpation. Usually the closer it gets to the fossa, the nearer the time of ovulation, but, again, follicles located at the poles may ovulate. In ovaries that are not too fibrous, the wedge-like protuberance of the mature follicle toward the fossa can be distinguished, and this has been suggested as an indication for approaching ovulation (58). Ovulation is recognized by a more or less sudden release of internal pressure, when not only the follicle but the entire ovary becomes flaccid and soft.

Transformation of the follicle into a corpus luteum after ovulation is rapid. According to Hamilton and Day (39), "within about 8 hours, the follicular cavity fills with a blood clot, and for 10 hours or so it is soft and pliable to the touch until 24-30 hours after ovulation when it is plum-like in consistency. It then becomes firmer and less conspicuous by palpation until by 4 or 5 days after ovulation, it is no longer palpable,

except that the ovary with the corpus luteum is normally about twice the size of the inactive ovary." The corpus luteum increases in size for about 4 to 5 days after ovulation, but it never reaches the size of the mature follicle (48). It reaches its maximal development at the 14th day of the cycle. The corpus luteum functions for 15 to 17 days, the period of diestrus in a normal heat cycle, and it persists anatomically in a relatively inactive state during the 2 or 3 cycles following its formation (35, 46).

Much effort has been directed toward determining the exact time of ovulation. It is well established that ovulation takes place spontaneously, and there is no experimental evidence to indicate that the act of copulation hastens the onset of ovulation. In view of the great variability in the duration of the estrous period and because of the variation in rate of follicular development, it is difficult to "fix" the time of ovulation. Assuming that an estrus of 4 to 8 days is normal, ovulation can be estimated to occur between the 3rd and 6th day. In short periods of less than 4 days, ovulation may occur within 24 hours after onset of estrus. Ovulation determines the end of estrus because between 24 to 72 hours after ovulation, the mare ceases to show symptoms of heat (29, 42). In other words, the hormones responsible for estrous behavior presumably are not present in sufficient amounts after this time. In exceptional instances, mares are reported to have shown estrous behavior for 6 to 11 days after ovulation (75). Inversely, ovulation has been observed as late as 5 days after the end of estrus (3, 29, 30).

Twin ovulations occur at a surprisingly high rate. Burkhardt (20) suggests that mares, known as "twinners," are predisposed to twin ovulations, a condition rare in the pony breeds. In some 80 mares whose ovaries were examined post-mortem in a slaughterhouse during the months of June and July, no less than 27% were found to have had double ovulations during the preceding estrous period (20). According to the same author, twinning occurs less frequently in the spring, again demonstrating the seasonal variation in fertility.

Other investigators report a much lower incidence of twin ovulations, that is, 3.8% (3, 75), and still less for twin pregnancies, 1.6 to 5.0%. The incidence of twin births is only 0.5 to 1.5% (4, 15). Curiously, Williams (70) attributed a high incidence of twinnings to pathological conditions in the uterus.

The ovaries do not necessarily ovulate alternately with successive heat periods; the left ovary is apparently more active than the right (3, 4).

B Changes in the Genital Tract

The genital tract undergoes definite changes during the cycle which are noticeable by visual and manual observation. These changes are correlated to corresponding changes in the ovary. They can be, and in practice are, utilized for detection of estrus and the optimal time for mating. Considerable variation exists, however, and the transition from one phase to the other is not as clear-cut as in laboratory animals. Thus, the changes in the reproductive tract are not always dependable indicators of ovarian activity. During the early part of the breeding season, with its pseudoestrous heat periods, "vaginal estrus" is associated with intense sexual desire, but ovarian function is incomplete (59).

During proestrus, the labial folds become loose, the vulva pendulous and vascular, especially in multiparous mares. During estrus, vulvar tone increases and the labia are swollen. Rhythmic contractions of the labia during teasing with the stallion, exposing the clitoris, together with hyperemia, are the most manifest indications of estrus. Similar contractions of the labia after urination are not indicative of estrus.

The changes in the vagina are quite definite in the healthy mare. Use is made of the appearance of the vaginal epithelium for detection of estrus in mares that fail to show estrus with a teaser stallion. During anestrus, and during late diestrus, the walls are partially glued together by a sticky, grayish secretion that makes the introduction of an unlubricated speculum or arm difficult. The epithelium has a dull, anemic appearance and is rough to the touch.

With approaching estrus, the adhesive quality of the vaginal secretion is less pronounced and in the anterior part a small amount of slimy mucus can be found. The walls of the vagina become more vascular and velvety.

With onset of estrus, when the mare shows definite receptivity to the stallion, the phenomena noticed during proestrus become more intense. Considerable amounts of a thin, yet clear mucus accumulate in the vagina, but may be absent in fully receptive mares because it may be expelled. It is partially of cervical origin. Its presence and degree of viscosity are the most dependable indicators for the presence of a mature follicle and approaching ovulation. In mares that remain in estrus for protracted periods the flow of mucus does not continue to the same extent and the vaginal walls tend to become dry and sticky.

Very significant and, therefore, useful are the changes in the cervix. When the mare is not in heat, this organ is rather firm, and has the shape of a dull cone. Frequently, the protruding cone lies on the floor of the vagina, and is covered by a dry, gummy secretion. Although the cervix

is closed, it is rarely as tightly constricted as in the diestrous cow. During proestrus, secretory activity increases and more and more fluid accumulates around its base. During estrus, and particularly during the stage close to ovulation, the position of the cervix shifts posteriorly. The cervical muscle tone is now characterized by its tactile sensitivity. It becomes erectile so that on being palpated it changes within minutes from a state of complete relaxation, resembling a rosette with rather loose folds around the os uteri, into a firmly constricted cone. During this phase, distinct, grasping contractions of the cervical sphinctor muscle can be felt. These contractions probably occur also during copulation and may permit close apposition of the expanded glans penis to facilitate deposition of the ejaculate into the uterus. Relaxation of the cervix is especially pronounced in mares during foal heat, but in this instance the contractions following copulation may not occur. In mares that are bred too early in the estrous period, the cervix does not open sufficiently and semen may be deposited in the vagina. In a mare with a healthy cervix, bred during the state of full estrous activity, no semen or only the residual portion of the ejaculate is found in the vagina after copulation. Following estrus, the healthy cervix returns to a less responsive resting state, and between the fifth and tenth day of the interestrous period it is closed and contracted.

The changes in the uterus during the cycle are not as easily detectable as in the rest of the reproductive tract. During diestrus it is rather rigid and contracted. In the estrous period it is more elastic and the body and horns appear full, firm, and turgid. They stay in this condition until ovulation, after which the uterus becomes limp and flaccid (31). Hammond (44) states that this change is sufficiently marked to be used for diagnostic purposes in determining the occurrence of ovulation.

The described changes in the reproductive tract also occur in the mare during silent heat. The mechanism of this condition is not known. In line with postulations put forward by Asdell (5), these mares may have a threshold level of responsiveness to estrogen stimulation much higher than in the normal mare, or, inversely, in them the nervous system is refractory to estrogen (25).

C. *Histological Changes in the Reproductive Tract*

Andrews and McKenzie (3) showed that the restoration of the uterine epithelium after foaling is rarely complete at the time of foal heat; in only 10% of the mares coming into heat on or before the tenth day after foaling is the endometrium fully comparable to that of a non-foaling mare on the first day of estrus. Complete restoration is usually accomplished 13 to 25 days after parturition. Before restoration there

is a lack of epithelium over large areas of the uterine mucosa, and degenerating glandular epithelium is seen. The histological changes in the reproductive tract are described in detail by Hammond and Wodzicki (46) and by Andrews and McKenzie (3), and are reviewed by Asdell (4) and Eckstein and Zuckerman (36).

The vaginal smear is not distinctive during estrus. No cell type is characteristic for any stage of the estrous cycle. Cornified and nucleated epithelial cells and leucocytes are usually present during all phases of the estrous cycle (3). Russian workers had, at one time, claimed that during estrus the vaginal smear consisted predominantly of leucocytes. Mirskaya and Salzman (66), on the other hand, reported that during proestrus the smear contained epithelial cells and during estrus, cornified cells and leucocytes. Cornified cells persisted until metestrus when epithelial cells and leucocytes became predominant (64).

Andrews and McKenzie (3) summarized the findings of their detailed investigations as follows:

"The epithelium of the vaginal mucosa reached its greatest height during estrus and a minimal thickness was usually recorded between the 5th and 15th day of interestrus.

"Cornification of the superficial layers of the vaginal epithelium was never marked but tended to be more prominent during estrus than at any other time.

"During the estrual cycle, leucocytes were most abundant in the stroma and vaginal epithelium at the time of estrus, and least numerous between the 5th to 10th day of interestrus.

"Stromal blood vessels were most congested during estrus, least prominent at about the 10th day of interestrus, and comparable to the interestrual condition during early and later pregnancy.

"During the sexual cycle, the height of the uterine surface epithelium was usually greatest during the latter stages of estrus and the first 5 to 8 days of interestrus. The minimal height was usually observed between the 10th and 15th day of interestrus.

"The diameter of the uterine glands and the height of the glandular epithelium were greatest between the 3rd day of estrus and the 5th day of interestrus and were least during the 6 to 7 day interval prior to the onset of heat.

"Leucocytes were present in large numbers during the period of involution of the endometrium. They were most numerous during estrus and least prominent at about the 10th day of interestrus.

"The stromal blood vessels were prominent prior to foal heat and during the cycle, were most congested at the time of estrus and least congested at about the 10th day of the interestrual period."

The cyclic changes in the histological picture of the cervix were described by Hammond and Wodzieki (46). During estrus the secretion of mucin by lining epithelial cells and by epithelial cells of the cervical glands contribute to the estrous flow of thin mucus. After ovulation these cells become cuboidal. Around the 8th day postestrus their secretory activity is low (3).

D. pH of Vaginal Secretions

Inasmuch as semen deposition during copulation takes place in the uterus through the open cervix, the pH of the vaginal secretion in the mare is unimportant. Andrews and McKenzie (3) found slight variations throughout the cycle, nonetheless. In general, the vaginal mucus is more alkaline prior to ovulation and less so following ovulation and during diestrus. The lowest vaginal pH occurs between the 5th and 10th day of diestrus.

The cervical mucus is slightly less alkaline, and, in some cases, it was slightly acid.

IV. THE BEHAVIORAL PATTERN OF THE CYCLIC MARE

Since hand breeding is widely practiced in present-day breeding enterprises, the correct interpretation of the sexual behavior and its correlation to physiological events within the mare is of great importance for: detecting mares ready for service, selecting the optimal time for breeding, and checking for recurrence of estrus.

In the mare, the transition in behavior from aversion toward the stallion during diestrus to sexual receptivity is not as abrupt as in other farm animals. The degree of expression varies from mare to mare. Also, the degree of intensity of mating desire is by no means an infallible indicator for the physiological state of the ovaries. During the transitional period from anestrus to the true breeding season in the early spring mares frequently express a very intense mating desire, yet their fertility is correspondingly low. Some mares, on the other hand, exhibit very low intensity of mating desire during the breeding season, even though ovulation occurs.

Full sexual receptivity and desire to mate is revealed by the rhythmic contractions of the labia and discharge of the typical, thin estrous secretion. Jennets show receptivity in a more pronounced manner by assuming a stance similar to lordosis, at the same time making gnashing motions with their mouths and folding their ears back on the neck. Mounting of other females, as is characteristic in cows, occurs in jennets but rarely in mares. Many estrous mares will seek the company of other

mares, however, and behave in a manner similar to that shown when in the presence of a stallion.

Most mares exhibit an increase in desire as the follicle matures, but individual variation is great. A mare with a small follicle may show heat with equal intensity to one with a large follicle.

Another feature that also can be used for determining the presence of estrus is the behavior of the stallion. A good teaser stallion responds to a mare in heat by becoming more excited. If the mare is not in heat, he soon loses interest. Possibly, stimulation is by a specific odor emanating from the mare. If this is true, its site of origin may be in the region of the flank, not around the genitalia.

After reaching the stage of highest sexual excitement, usually coinciding with ovulation, the symptoms abate within 2 or 3 days, with gradual loss of interest until the diestrous stage of resistance to the stallion is reached. Expression of nonreceptivity, too, varies from mare to mare. Some become aggressive toward the stallion, some are merely disinterested, and some continue to be mildly interested in the stallion throughout diestrus, giving a confusing picture. In earlier times, untrained personnel used the malpractice of vigorous teasing on the premise that a mare could be teased into heat and acceptance. The decision concerning the time of mating should be based upon the condition of the ovaries as determined by rectal palpation as well as on behavior.

Subnormal estrous behavior with apparently normal ovarian changes characteristic of estrus is a frequent occurrence. McKenzie (62) reported that in a mare population studied by him, 15% passed through an entire breeding season without showing any signs of heat. That these mares were capable of reproducing was demonstrated by the fact that 68% became pregnant with artificial insemination after their estrous cycles were established by palpation of the ovaries and observations of the reproductive tract. Periods with physiological or silent heat also occur intermittently between regular heat periods.

Andrews and McKenzie (3) presented in graphic form the cyclic behavior of mares, using a scale for the intensity of "psychological" reactions graded in 8 steps ranging from "very receptive" to "very actively resistant," which demonstrates the fluctuations of sexual behavior throughout the cycle and the variations between individual mares.

V. ADAPTATION OF THE BREEDING PROGRAM TO CYCLIC EVENTS

Because of the great variation in responsiveness of the reproductive system of individual mares to seasonal factors, it seems advisable to establish before the start of breeding operations the degree of reproductive preparedness of each mare. At the present time, the only available

criterion for this is the type of estrous cycles. Normal cycles with short estrous periods during the prebreeding season would reveal the completely polyestrous individuals that are either entirely independent from extraneous stimuli or are highly responsive of them. They are ready to be bred as soon as it is practical. Those that are less responsive and take longer to reach full reproductive capacity, as signified by the degree of estrous rhythmicity, cannot be bred successfully until their reproductive system gets into balance.

Hormonal therapy has become a valuable aid for achieving a high degree of reproductive performance both by terminating anestrus and by correcting aberrations during the cycle. Although gonadotropin administration has not proved effective in mares in deep anestrus, shallow anestrus can be overcome by administration of gonadotropins. Subcutaneous administration of 1000 to 2000 IU of equine gonadotropin has been reported to be effective as a supplement to the lagging pituitary (4, 6, 9, 22, 31, 44, 45). Another approach is to stimulate the mare's own pituitary by the administration of estrogen. With this method, however, one must guard against pituitary suppressing effects by excessive dosage. Whereas stimulation of follicular growth and of uterine and cervical functions have been obtained with doses of 5 to 15 mg. dienestrol or stilbestrol, doses of 20 to 25 mg. have been reported to cause inhibitory effects. These effects are manifested by pronounced ovarian derangements and loss of estrous rhythmicity of long standing in some mares. Others exhibit protracted estrus and still others revert into an anestrus condition (9, 13, 19).

Follicles that fail to ovulate may cause protracted periods of estrus. These follicles may be stimulated by intravenous administration of 1,000 to 2,000 IU of chorionic gonadotropin. Ovulation occurs within 20 to 60 hours depending on the condition of the follicle at the time of injection (11, 12, 20, 28, 39, 45, 62, 65, 66). Administration of small amounts of estrogen in such cases is also effective (3, 19, 43).

Ovulation by hormonal means can be induced in normal estrous mares to synchronize ovulation with mating. The survival time of stallion sperm is not definitely known. It has been claimed that it can survive for as long as 4 to 6 days after ejaculation (31, 32, 75). Possibly some stallions produce sperm of exceptional viability but, on the basis of numerous investigations, it would appear that the survival period within the uterus is much shorter, 24 to 72 hours (7, 10, 39, 43, 45, 56). Although a few sperm may reach the ovulated ovum within 15 to 18 minutes after mating (41, 72), it takes about 8 hours for sufficient sperm numbers to arrive for effectual fertilization. Since the ovulated ovum remains fertile for only 4 to 20 hours, some mares may be bred too late in estrus, but

usually mares are bred too soon, especially if only one service is allowed during estrus

Hammond (43) said that the "fertility of matings gradually rises to a peak about 2 days before the end of estrus and then falls off sharply during the last 24 hours of heat to total sterility later," but successes have been reported with services delayed for 12 to 24 hours after ovulation (4) In mares with short heat periods of 1 to 3 days, service should be performed on the first day, in others on the 3rd or 4th day and again 48 to 72 hours later If estrus persists longer than 8 to 10 days, services should be discontinued until the next heat period (64)

REFERENCES

- 1 Aehnelt, E, and Plas, J, *Deut tierarzt Wochschr* 53, 10, 52, 84 (1946), *Animal Breed Abstr* 15, 233 (1947)
- 2 Aitken, W A, *J Am Vet Med Assoc* 70, 481 (1927)
- 3 Andrews F N, and McKenzie, F F, *Missouri Agr Expt Sta Research Bull* 329 (1941)
- 4 Asdell, S A, 'Patterns of Mammalian Reproduction' Comstock, Ithaca, New York, 1946
- 5 Asdell, S A, in "The Problem of Fertility" (E T Engle, ed), p 1 Princeton Univ Press, Princeton, New Jersey, 1946
- 6 Asdell, S A, in 'Progress in the Physiology of Farm Animals' (J Hammond, ed), Vol 3, p 743 Butterworths, London, 1957
- 7 Berliner, V R, *J Animal Sci* 1, 62 (1942)
- 8 Berliner, V R, in 'The Artificial Insemination of Farm Animals' (E J Perry, ed), Chapt 7 Rutgers Univ Press, New Brunswick, New Jersey, 1945
- 9 Berliner, V R, in The Artificial Insemination of Farm Animals (E J Perry, ed), 2nd rev ed, Chapt 9 Rutgers Univ Press, New Brunswick, New Jersey, 1952
- 10 Berliner, V R, in 'The Problem of Fertility' (E T Engle, ed), p 187 Princeton Univ Press, Princeton, New Jersey, 1946
- 11 Berliner, V R, *The Blood Horse* 49, 694, 768 (1955)
- 12 Berliner, V R, Cowart, F E, Means, R H, and Wright, J B, *Proc Am Soc Animol Production* 233 (1938)
- 13 Berliner, V R, and Scales, J W, *J Animal Sci* 3, 431 (1944)
- 14 Berliner, V R, Sheets, E W, Means, R H, and Cowart, F E, *Proc Am Soc Animal Production* 295 (1938)
- 15 Blakeslee, L H, and Hudson R S, *J Animal Sci* 1, 155 (1942)
- 16 Britton, J W, and Howell, C E, *J Am Vet Med Assoc* 102, 427 (1943)
- 17 Britton, J W, and Howell, C E, *Vet Med* 40, 264 (1945)
- 18 Burkhardt, J, *J Agr Sci* 37, 64 (1947)
- 19 Burkhardt, J, *Vet Record* 59, 341 (1947)
- 20 Burkhardt, J, *Vet Record* 60, 243 (1948)
- 21 Burri, K, *Schweiz Arch Tierheilk* 90, 323 (1948)
- 22 Cameron, H S, *J Am Vet Med Assoc* 100, 60 (1942)
- 23 Cusick, E, *Cornell Vet* 27, 187 (1937)
- 24 Cole, H H, in "The Problem of Fertility" (E T Engle, ed), p 71 Princeton Univ Press, Princeton, New Jersey, 1946

25. Cole, H. H., *Western J. Surg. Obstet., Gynecol.* 56, 503 (1948).
26. Constantinescu, C. K., and Mauch, A., *Ann. Inst. Natl. Zootech. Roumanie* 5, 9 (1936).
27. Cummings, J. N., *J. Animal Sci.* 1, 309 (1942).
28. Davison, W. F., *J. Agr. Sci.* 37, 287 (1947).
29. Day, F. T., *J. Agr. Sci.* 29, 459 (1939).
30. Day, F. T., *Vet. Record* 51, 1113 (1939).
31. Day, F. T., *J. Agr. Sci.* 30, 244 (1940).
32. Day, F. T., *J. Agr. Sci.* 32, 108 (1942).
33. Dukes, H. H., "The Physiology of Domestic Animals," Comstock, Ithaca, New York, 1943.
34. Eckstein, P., and Zuckerman, S., in "Marshall's Physiology of Reproduction" (E. S. Parkes, ed.), 3rd ed., Chapt. 2. Longmans, Green, London, 1956.
35. Eckstein, P., and Zuckerman, S., in "Marshall's Physiology of Reproduction" (E. S. Parkes, ed.), 3rd ed., Chapt. 4. Longmans, Green, London, 1956.
36. Eckstein, P., and Zuckerman, S., in "Marshall's Physiology of Reproduction" (E. S. Parkes, ed.), 3rd ed., Chapt. 6. Longmans, Green, London, 1956.
37. Coetzee, R., *Deut. tierarztl. Wochschr.* 43, 161 (1935).
38. Halasz, B., *Acta Agron. Acad. Sci. Hung.* 4, 151 (1954); *Animal Breed. Abstr.* 22, 290 (1954).
39. Hamilton, W. J., and Day, F. T., *J. Anat.* 79, 127 (1945).
40. Hammond, J., *Spec. Rept. 18th Congr. Intern. Agr. Budapest* (1934) (Reprint).
41. Hammond, J., *Proc. 1st Intern. Congr. Physiology and Pathol. Animal Reproduction and Artificial Insemination Milan* (1948). *Zootec. e Vet., Spec. No.* June (1948).
42. Hammond, J., *Fiziol. Zhur. S.S.S.R.* 21, 193 (1938).
43. Hammond, J., in "The Problem of Fertility" (E. T. Engle, ed.), p. 60. Princeton Univ. Press, Princeton, New Jersey, 1946.
44. Hammond, J., in "The Problem of Fertility" (E. T. Engle, ed.), p. 243. Princeton Univ. Press, Princeton, New Jersey, 1946.
45. Hammond, J., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), Vol. 2, Chapt. 21. Longmans, Green, London, 1952.
46. Hammond, J., and Wodzicki, K., *Proc. Roy. Soc. B130*, 1 (1941).
47. Hancock, J. L., *Vet. Record* 60, 679 (1948).
48. Harrison, R. J., *J. Anat.* 80, 160 (1946).
49. Howell, C. E., and Rollins, W. C., *J. Animal Sci.* 10, 789 (1951).
50. Jennings, W. E., *J. Am. Vet. Med. Assoc.* 116, 11 (1950).
51. Kellner, R., *Deut. landwirtsch. Tierzucht* 37, 762 (1933).
52. Kellner, R., *Z. Gestützkunde u. Pferdezucht* 29, 139 (1949).
53. Kem, *Tierzüchter* 1, 129 (1949). *Animal Breed. Abstr.* 17, 329 (1949).
54. Küpfer, M., *Rept. Vet. Research S. Africa* 13, 14, Pt. 2 (1928).
55. Lagerlöf, N., *Scensk Veterinärtidskrift* 41, 173 (1936).
56. Laing, J. A., *J. Agr. Sci.* 33, 64 (1943).
57. Laing, J. A., in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 3. Butterworths, London, 1957.
58. Lensch, J., *Irish Vet. J.* 11, 22 (1957); *J. Am. Vet. Med. Assoc.* 132, 209 (1958), abstract.
59. Mahaffey, L. W., *Australian Vet. J.* 26, 267 (1950).
60. Mauch, A., *Z. Zücht. Reihe B* 39, 31 (1937).
61. McGee, W. R., "Veterinary Notes for the Standard Red Breeder," United States Trotting Assoc., Columbus, O.

- 62 McKenzie, F F, *Proc Am Soc Animal Production* 98 (1940)
- 63 McManamny, L F, *Australion Vet J* 25, 274 (1949)
- 64 Milovanov, V K, "Principles of Artificial Insemination" Gosisdat, Moscow, U S S R, 1934
- 65 Mirskaya, L M, and Petropavlovsky, V V, *Problems Animol Husbandry U S S R* 4, 32 (1937)
- 66 Mirskaya, L M, and Salzman, A A, *Advances Zootech Sci (Moscow)* 1, 157 (1935)
- 67 Nishikawa, Y, Sugie, T, and Harada, N, *Bull Notl Inst Agr Sci (Jopon) Ser G, No 3*, 35 (1952), *Animol Breed Abstr* 22, 103 (1954)
- 68 Nishikawa, Y, and Yamasaki, Y, *Jopon J Zootech Sci* 19, 119 (1949), *Animol Breed Abstr* 18, 368 (1950)
- 69 Nishikawa, Y, and Yamasaki, Y, *Jopon J Zootech Sci* 20, 28 (1949), *Animal Breed Abstr* 18, 369 (1950)
- 70 Nishikawa, Y, and Yamasaki, Y, *Jopon J Zootech Sci* 20, 80 (1949), *Animal Breed Abstr* 19, 35 (1951)
- 71 Quinlan, J, Van Rensburg, S W J, and Steyn, H P, *Onderstepoort J Vet Sci Animol Ind* 25, 105 (1951), *Animal Breed Abstr* 19, 428 (1951)
- 72 Rice, V A, "Breeding and Improvement of Farm Animals," 3rd ed McGraw-Hill, New York, 1942
- 73 Rollins, W C, and Howell, C E, *J Animal Sci* 10, 797 (1951)
- 74 Satoh, S, and Hoshi, S, *J Japan Soc Vet Sci* 11, 257 (1932)
- 75 Trum, B F, *Cornell Vet* 40, 17 (1950)
- 76 Uppenborn, W, *Z Zucht Reihe B Z Tierzucht Zuchtungsbiol* 28, 1 (1933)
- 77 Van Rensburg, S W J, and Van Herden, J S, *Onderstepoort J Vet Research* 26, 285 (1953)
- 78 Wellmann, O, *Landwirtsch Jahrb* 39, 409 (1910), quoted by Dukes (33)
- 79 Williams, W. L., "The Diseases of the Genital Organs of Domestic Animals," 3rd ed, W L Williams, Ithaca, N Y, 1943

CHAPTER 9

The Estrous Cycle of the Ewe and Doe

T. J. ROBINSON

	<i>Page</i>
PART 1: The Ewe	292
I. Introduction	292
II. The Sexual Season	292
III. The Estrous Cycle	295
IV. Cyclic Changes in the Reproductive Organs	296
A. Ovary	296
1. Follicular Maturation and Ovulation	296
2. Formation of the Corpus Luteum	298
B. Oviduct	298
1. Physiological Changes	298
2. Ovum Transport	298
3. Sperm Transport	299
C. Uterus	299
D. Cervix	299
E. Vagina	300
F. Vaginal Smear	300
1. Cyclic Changes in General Smear Pattern	300
2. Cyclic Changes in Constituents of Smear	300
3. Definition of Estrogenic Activity	303
4. Practical Uses	304
V. Endocrine Control of the Cycle	305
A. Cyclic Changes in Hormone Production	305
1. Anterior Pituitary Hormones	305
2. Gonadal Hormones	307
B. Control of Ovulation	309
C. Control of Estrus	312
D. Control of Changes in the Reproductive Tract	323
VI. Artificial Control of the Cycle	324
A. Economic Significance	324
B. Control in Anestrus	325
C. Control in the Breeding Season	325
1. Number of Ovulations	325
2. Time of Ovulation	320
D. Conclusions	328
PART 2: The Doe	328
VII. Introduction	328
VIII. Characteristics of the Cycle	328
References	329

Part I: The Ewe

I. INTRODUCTION

The ewe is a seasonally polyestrous animal in which the number of annual consecutive cycles ranges from 1 in the primitive mountain sheep to 20 or more in the Merino (68, 69, 86, 107). Seasonal variations in fertility exist even in those breeds which exhibit little, if any, anestrus period. Inability to breed British mutton and long-wool breeds for a major part of the year presents an acute problem of practical sheep husbandry. Hence the estrous cycle of the ewe has been the subject of intense study since the publication of Marshall's original work in 1903 (85).

II. THE SEXUAL SEASON

The number of recurrent cycles characteristic of the various types and breeds of sheep appears to be related to the severity of the environment in which their progenitors evolved (51, 55). The Scotch Black-faced and the Merino are extreme examples of domesticated sheep. The former evolved in a harsh environment in which only lambs born in a restricted period of the spring had any chance of survival. Selection therefore has been toward a type of ewe with a very short breeding season. No such strong natural selection operated on the progenitors of the modern Merino sheep. They evolved in a relatively benign environment in which lambs born at any time of the year had a chance of survival. Nevertheless, even such sheep have an intrinsic annual rhythm of reproductive efficiency, which reaches its peak in the autumn months (2, 68, 69, 146, 147, 148). In the British mutton and long-wool breeds this rhythm is expressed in a manner intermediate between that of the Merino and the mountain breeds: anestrus is a period of relative rather than absolute quiescence, during which changes in the ovaries and accessory reproductive organs, unaccompanied by overt estrus, commonly occur (115).

The effect of environmental factors on the sexual season is considered in other chapters, and thus only brief reference will be made here. Diurnal light-dark rhythm appears to be the most fundamental environmental factor (51, 60, 134, 154, 155, 156). Figures 1 and 2 illustrate the relationship between the length of daylight and sexual activity in several breeds of sheep. Other environmental factors modify this photoperiodic control. Faulty nutrition is reported to delay the onset of the sexual season in the Merino (71, 107). Scotch Black-faced ewes experience 5 to 9 estrous cycles in a favorable environment, as compared with 2 or 3 in the highlands (51). On the other hand,

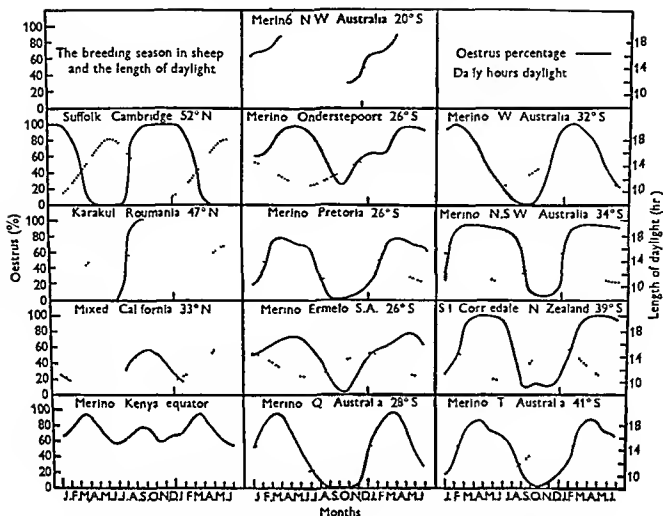


FIG 1 The relationship between the breeding season and the length of daylight at different latitudes From Hafez (51)

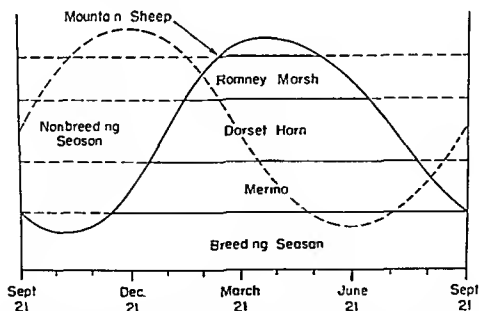


FIG 2 Diagrammatic representation of the relationship between the breeding and nonbreeding seasons of several breeds of sheep and the photoperiodic rhythm ——— curve of reproductive activity, - - - - - curve of daylight hours Dates refer to Southern Hemisphere

numerous authors report difficulty in modifying the time at which sexual activity begins in British lowland breeds by altering the plane of nutrition (4, 13, 16, 87, 138). Earlier evidence to the contrary (68, 82, 155), a cool climate appears to influence favorably initiation of sexual activity in sheep (32). Psychic factors may be involved. Thus, a definite anestrus period is shown in the spring by Merino ewes in constant association with vasectomized rams (69). This observation is at variance with overwhelming field evidence that introduction of rams to segregated Merino ewes in spring results in reasonable lambing performance. Introduction of the ram into an anestrus flock appears to hasten the

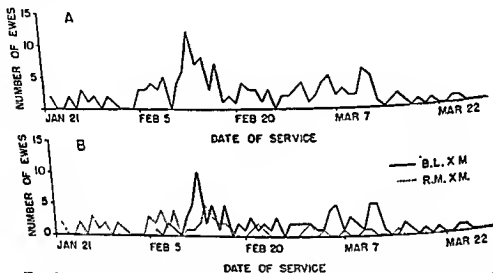


FIG. 3. Effect of introduction of the ram at the start of the breeding season on the incidence of estrus. (A) All ewes in the flock; (B) Differential effect on two types of crossbreed, namely, Border Leicester \times Merino and Romney Marsh \times Merino. Rams were introduced on January 21, by which date Romney Marsh \times ewes had commenced their intrinsic estrous rhythm. Border Leicester \times ewes were still in anestrus, and exhibited the characteristic psychic response to the unaccustomed presence of the ram. From Papadopoulos and Robinson (100).

sexual season (51, 100, 111, 130, 131, 139). As shown in Fig. 3, manifestation of this phenomenon appears critically dependent upon the ovarian condition at the time. Intrinsic, as well as extrinsic, environmental factors may influence either the onset or the duration of the sexual season, as indicated by the effect of age (21, 56, 81, 84, 128).

The first ovulation of the normal breeding season of the ewe, as with gonadotropin-induced or spontaneous ovulation in anestrus, is not normally accompanied by overt estrus (19, 20, 44). This is a phenomenon usually considered peculiar to the ewe, but which has also been reported to occur commonly in the goat (23) and rarely in the cow (54). Such

"silent heats" have also been reported toward the end of the breeding season (84). The former type invariably ensues when ovulation occurs in the absence of a corpus luteum from a previous cycle (115), while it appears probable that "silent estrus" at the end of the breeding season is due to an insufficiency of estrogen (see below).

III. THE ESTROUS CYCLE

In 1900, Hcape (63) described the phases of the cycle as proestrus, estrus, metestrus, and diestrus. This terminology is still in general use but the suffix "us" has generally replaced "um" in modern literature. The characteristics of the cycle of the ewe have been described (44, 81, 86, 128) and the literature reviewed (5, 84) by a number of authors. The phases of the cycle discussed below are as defined by Asdell (5, p. 20) for cycles which include an active luteal phase and in which metestrus is defined as the brief period of formation of the corpus luteum, and diestrus as the relatively long period of luteal activity.

The duration of the cycle is remarkably constant, with a mean of 16.4 to 17.5 days (modal range, 14-19 days). Merino and Rambouillet ewes have cycles about one day longer than Scottish and Navaho ewes, with the British breeds intermediate (Table I). Williams *et al.* (149) report that early and late in the breeding season the number of abnormally short (< 14 days) or long (> 19 days) cycles is dispropor-

TABLE I
LENGTH OF THE ESTROUS CYCLE IN SHEEP (5)

Breed	Length of cycle (days)	Range (days)	Remarks
Scottish	18.4 \pm 0.8	15-18.5	Mode, 18.5
Navaho	16.44 \pm 0.10		
Hampshire	18.5		Flushed
Shropshire and Hampshire	16.7		Mode, 17; 88% from 15.5-17.5 days
Romney	16.7		Mode, 17; 89% from 14-19 days
Merino	16.8	12.5-18.5	Mode, 16.5
Tzigai	17	16-21	
Dorset	17		
Merino	17		
Romney	17.0	14-39	91% from 15-18 days
Hampshire	17.2		Unflushed
Merino	17.3		Mode, 17; grades
Merino	17.4 \pm 0.08	6-27	Mode, 17; SD, 1.8; 85% from 16-19 days
Rambouillet	17.5	13-21	
Tzurcana	17.5	15-20	

tionately large. Not all authors agree (21). Part of this discrepancy is probably due to different methods of determining estrus. The more tedious method of yarding twice daily and teasing, as used by Cole and Miller (21), is probably more accurate than the more commonly used method of turning in vasectomized raddled rams with the ewes. The latter method has the advantage of practicability for large-scale field observations.

Proestrus in the ewe, as in other species, is relatively ill-defined. There is rapid growth of the definitive follicle(s), accompanied by increased estrogen secretion which stimulates proliferative changes in the accessory organs. During a short period, usually less than an hour, the ewe evidences increased restlessness, tail wagging, and courting of the ram without acceptance (84, 107, 109).

Extreme differences in the duration of overt estrus, ranging from 3 to 84 hours, are reported both between and within breeds. These reports demand careful interpretation, since many factors, such as age, relative stage of the breeding season, nutritional status, and libido of the ram, can affect the observed period of receptivity. Generally speaking, ewes can be expected to accept the ram for a period of 24 to 36 hours. Young sheep tend to have shorter heats than old sheep; they mate less frequently and to fewer rams (66, 72). Sterile service may possibly shorten slightly the duration of estrus. Heat shows no tendency to appear more frequently at any specific time of the day (84). According to Kelley (68), vaginal and perineal swabs from estrous ewes attract the ram when applied to pregnant animals, suggesting that the olfactory sense is important in mating behavior.

During the early stages of metestrus, the ram is attracted but not accepted. As defined above, metestrus is roughly the period during which the influence of estrogen is declining. This is followed by a period of apparent sexual quiescence—diestrus—during which the influence of the corpus luteum is predominant. The cyclic activities of the ewe have not been followed sufficiently closely to permit accurate delimitation of any phase of the cycle other than estrus.

IV. CYCLIC CHANGES IN THE REPRODUCTIVE ORGANS

A. Ovary

1. Follicular Maturation and Ovulation

According to Cole and Miller (21), no new ova are produced from the germinal epithelium during the sexual life of the sheep, although they are produced from the neogenic layer below the tunica albuginea. The prepubertal ovary is small and contains few follicles. Pre-

sumably waves of follicle growth and atresia occur prior to the first ovulation, as in the anestrus mature ewe in which quiescence is only relative (21, 113).

The cyclic ovary is characterized by a follicular phase which spans proestrus and estrus—3 to 4 days—followed by a luteal phase of some 13 days.

Except at the beginning of the breeding season, the proestrous ovary is characterized by (a) one or more developing follicles which are destined to rupture toward the end of the ensuing heat period, and (b) a degenerating corpus luteum from a previous cycle. Both are essential prerequisites for normal estrus. The ripening follicles are large and turgid and generally easily distinguishable except in maiden ewes in which they may be less than 5 mm. in diameter.

Growth and rupture of the follicle during estrus have been described by several authors (21, 80, 83, 84, 107) and the literature reviewed by Asdell (5). Growth is rapid. All the follicle layers become thin, vascularity increases, and the follicle swells beyond the surface of the ovary and becomes dome-shaped. In the mature ewe it is now about 10 mm. in diameter. About 1 hour before ovulation a small, round, transparent ovulation point appears, within which a cone forms immediately prior to ovulation. This ruptures and the follicular fluid flows out—it rarely spurts—and the follicle gradually collapses. Initially thin, the fluid becomes viscous within 2 or 3 minutes.

Ovulation is spontaneous and independent of coitus. Observers who report a slightly accelerated termination of estrus when mating occurs stress that the time of ovulation is unaffected (84). Estimates of this time, relative to the time limits of heat, vary somewhat, due no doubt to the labile nature of estrus and, perhaps more importantly, to the lack of sufficient care in determining the initiation of estrus. The only reliable conclusion to be drawn from the many reports is that ovulation occurs at about the end of estrus (3, 13, 84, 107, 132).

The number of ovulations—1 or 2, occasionally 3, rarely 4 or more in most breeds—undoubtedly constitutes a factor limiting fertility (53). Characteristic breed differences exist and are reflected in fecundity, as tabulated by Asdell (5). These breed differences notwithstanding, the number of ovulations is considerably influenced by age, stage of the breeding season, and plane of nutrition. The number of ova shed at each heat period reaches a maximum between 3 and 6 years (84), and some time after the start of the breeding season (7, 44, 56, 84). The time-honored practice of "flushing" ewes by placing them on a high plane of nutrition shortly before joining appears to operate by increasing

the number of ova shed. El Sheikh *et al.* (39) compared the number of ovulations of two groups of ewes joined and slaughtered together after having been kept for 6 months on a high (roughage and grain) and a low (roughage only) plane of nutrition, respectively. The average numbers of ovulations were 1.81 and 1.27, and 1.66 and 1.01, respectively, in the 2 successive years of the experiment.

2. Formation of the Corpus Luteum

There is no hemorrhage into the cavity of the ruptured follicle, but a small clot may close the opening. The follicle walls grow in and the cavity is more or less completely closed some 30 hours after ovulation. The corpus luteum is developed from both granulosa and theca interna cells, but the lutein cells are mainly of granulosa origin as those of the theca rapidly degenerate.

The corpus luteum reaches its maximum size at about 6 to 8 days. It is then reddish-pink in color, but gradually becomes paler as diestrus advances. After the 14th day fatty degeneration is rapid (107). The lutein cells measure 25–30 μ at first, and gradually increase in size until degeneration commences (143).

B. Oviduct

1. Physiological Changes

The fimbriated funnel into which the ovum is shed closely invests the ovary at estrus (107). The ciliated columnar epithelium and the cilia increase in height coincident with the development of the corpus luteum. This occurs first at the fimbriated end and later in the mid-tubal area. Cytoplasmic extrusions occur during diestrus (14, 50).

Histochemical techniques suggest that mucoprotein or "neutral mucopolysaccharide" is secreted by the nonciliated epithelial cells during proestrus and estrus (47, 50). The pH rises at this time from the diestrus value of 6.0–6.4 to 6.4–6.6 in proestrus and to 6.8–7.0 in estrus and metestrus (48). It seems reasonable to assume that these changes are associated with the production of an optimal environment for sperm and ovum transport and for fertilization.

2. Ovum Transport

The ovum measures 142–153 μ in diameter and its zona pellucida, 15 μ (17, 49). The first polar body is usually extruded just before or just after ovulation (21). The cumulus cells are nonpersistent and fertilization must occur within 15 to 24 hours of ovulation, apparently in the upper third of the oviduct (16, 45). First division occurs some 24–36 hours after ovulation and subsequent cleavage follows fairly

rapidly; 3- to 4-cell and 8-cell tubal ova can be recovered at 26-32 and 42-48 hours postcoitus respectively, and 16-cell at 65 hours (16, 46, 151). The ovum enters the uterus at about 70 hours, usually in the 8- or 16-cell stage (6, 16, 151).

Since ciliated cells extend the whole length of the oviduct (84), ovum transport is probably similar to that in the rat, namely, by a combination of peristalsis and ciliary action (97, 101, 106, 152).

Ovum transport is greatly accelerated when ewes are multiple-ovulated with equine gonadotropin (PMS). If the dose is excessive (e.g., 2000 I.U.) fertilization may be impaired (114), but segmentation of those eggs which are fertilized, although temporarily slowed down, is normal by the 9th day (153).

3. *Sperm Transport*

The rate and efficiency of sperm transport appears to be critically related to the condition of the reproductive tract, and varies greatly from ewe to ewe. This may account for the wide discrepancies in the reported times of transport of sperm from the vagina to the oviduct, which range from 6 to 25 minutes (132, 133) to from 5 to 8 hours (25, 29, 45, 103).

The cervix, which is a formidable barrier (25, 27, 28, 29), appears to act as a reservoir for viable sperm, and survival times of up to 78 hours are reported (28, 108). Estimates of survival time in other parts of the tract are: vulva—3 hours; vagina—6 to 12 hours; uterus and oviduct—9 to 30 hours.

Studies by Dauzier (22) suggest that transport, particularly through the cervix, is effected by spermatozoal motility.

C. *Uterus*

The original observations of Marshall (85) on cyclic uterine changes have been confirmed and considerably extended (14, 21, 84). Edema and increased vascularity are evident in both cotyledonary and inter-cotyledonary areas at estrus and metestrus. This is followed by increases in the growth and coiling of the uterine glands, of the height of the basal epithelial cells, and in mid-cycle by a folding of the free surface of the epithelium. Leucocytes are present at all stages, but appear in greatest numbers late in diestrus.

D. *Cervix*

The cervical glands are relatively simple in anestrus (21). The mucin of the cervical secretion of proestrus and estrus builds up in the cervical cells during diestrus (84).

E. Vagina

Cyclic changes in the vagina, while not particularly marked, are generally similar to those observed in other species (44, 62, 84). Growth of the epithelium is continuous and is slightly accelerated during estrus. In late estrus and in metestrus desquamation occurs and as many as 4 or 5 cell layers may be shed. This shedding is regional, never general (44).

Lymphocytes and leucocytes are usually present in the epithelium, but the incidence of the latter is greatest from early to mid-diestrus. Edema of the vaginal stroma is characteristic of late proestrus and early estrus (4).

F. Vaginal Smear

1. Cyclic Changes in General Smear Pattern

At estrus there is a copious flow of very thin mucus containing relatively few cells. Leucocytes are almost entirely absent. By the following day (metestrus) the volume of mucus has decreased markedly and its viscosity increased. Smears now contain large quantities of desquamated cells, many of which are cornified, and have a "cheesy" appearance. Such smears persist for 2 or 3 days, during which period the number of leucocytes progressively increases. These persist until the next proestrus. In mid- and late diestrus (days 8 to 14) smears are scanty and consist mainly of leucocytes and cellular detritus with a few squamous cells (4, 21).

These changes, described in general terms by earlier authors, have recently been defined in terms of the various components of the smear (125). These components are (a) mucus; (b) leucocytes; (c) squamous and nucleated epithelial cells; (d) cornified cells; and (e) cellular detritus.

2. Cyclic Changes in Constituents of Smear

Mucus is invariably present; its quantity and viscosity vary enormously with the stage of the cycle. Estrus—"silent" or overt—is accompanied by a copious flow of thin cervical mucus which shows the phenomenon of "crystallization," i.e., the production of a fernlike pattern when spread on a dry glass slide (79). After persisting for a day it rapidly decreases in volume and increases in viscosity. During diestrus it is scanty and usually thick (Fig. 4).

The appearance and disappearance of leucocytes is not as clear-cut as in the rodent or the bitch (125). They never entirely disappear from the smear at any stage of the cycle of many ewes. Nevertheless,

distinct rhythmic fluctuations occur in the relative numbers present in individual smears (Fig. 5). Relatively few appear on the day of estrus and for 2 or 3 days thereafter. They reach their maximum incidence by the 5th or 6th day, from whence they appear consistently until the day of the next estrus.

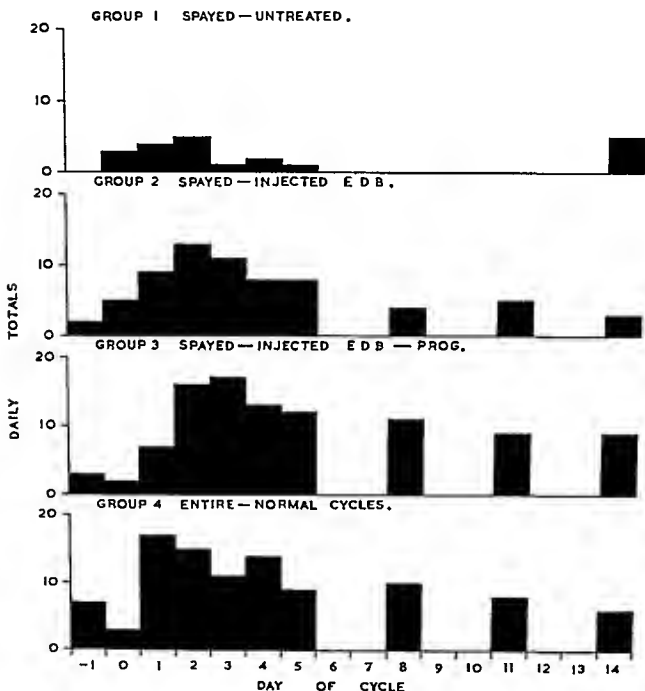


FIG. 4. Cyclic changes in the viscosity of mucus in vaginal smears of the ewe. The figure illustrates the number of smears which contained mucus classed as "thick" on successive days of the estrous cycle in entire ewes (Group 4) and in spayed animals untreated (Group 1), injected with estrogen alone (Group 2), or with estrogen followed by progesterone (Group 3). Estrus = day 0. Estrogen (Estradiol benzoate, EDB) injected on day 2. Progesterone (prog) injected in Group 3 on days 1 to 12. $n = 20$. From Robinson and Moore (125).

numbers of nucleated epithelial and squamous cells are an invariable sequel to estrus. The former type occasionally appear in estrus and are generally present in the smear of late estrus, by which time they are accompanied by large numbers of squamous cells. In the next 2 or 3 days the latter cell type tends to dominate the smear

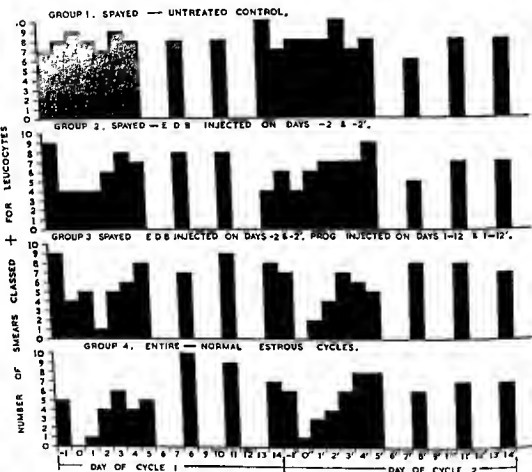


FIG. 5. Cyclic appearance and disappearance of leucocytes in the vaginal smear of the cow. The figure illustrates the number of smears classed as positive for leucocytes in two successive cycles. Group treatments and reference are as in Fig. 4. $n = 10$.

picture, usually but not invariably accompanied by many cornified cells. Thenceforth the numbers decrease rapidly and by mid- to late diestrus, smears are usually scanty and relatively free of intact cellular elements.

Cornified cells usually appear in small numbers on the day of estrus, but some may be present in proestrus. Their number increases rapidly and reaches a maximum 2 days after estrus, by which time about 50%

of smears may be expected to consist almost entirely of a dense cheesy mass of squamous and cornified cells.

Several additional, rather ill-defined cell types occur. For example, spherical epithelial cells are of two types. Large nucleated cells, presumably of vaginal origin, with light-staining cytoplasm and clearly defined nucleoli, predominate. Smaller cells with dark-staining nuclei are occasionally observed, and are probably analogous to cells described for the cow by Hansel *et al.* (59) and believed to be of uterine origin. Their presence is of no value in determining the stage of the cycle.

The smear of late diestrus is characterized by the presence of degenerating squamous cells, accompanied by a few nucleated epithelial cells and some leucocytes.

3. Definition of Estrogenic Activity

Robinson and Moore (125) have recently developed a system of classifying smears for estrogenic activity, based on a comparison of the smear pattern of intact cyclic ewes and that of spayed ewes treated with physiological doses of estrogen alone or in combination with progesterone. The system, summarized in Table II, involves the daily col-

TABLE II
SYSTEM OF CLASSIFICATION OF SMEARS WHICH CONTAIN CORNIFIED CELLS (125)

Degree of cornification		Classification for major cell types				Over-all smear classification
		Leucocytes	Nucleated epithelial	Squamous	Cornified	
Grade	Description					
1	Fully cornified	—	—	—	+	+
2	Almost fully cornified	—	—	+	+	+
3	Largely cornified	—	+	+	+	+
4	Slightly cornified	+	+	+	+	—

lection of smears for 4 days commencing on the day of estrus, followed by staining and systematic classification for each of 4 cell types, namely, leucocytes, nucleated epithelial, squamous, and cornified cells. For each cell type there are two classifications: (a) none to very few, and (b) few to many. These are graded — and +, respectively. All smears are examined for leucocytes first, and all graded + are discarded. The remainder are then classed for the other 3 cell types; any graded + for cornified cells (regardless of other cell types) are classed positive for estrogenic activity.

For the purposes of formal statistical analysis, smears are classed simply as + or —. The distribution of the grades of smear shown in Fig. 6, examples of which are reproduced in Fig. 7, is of interest none-

indicates the sequence of changes which occurs during

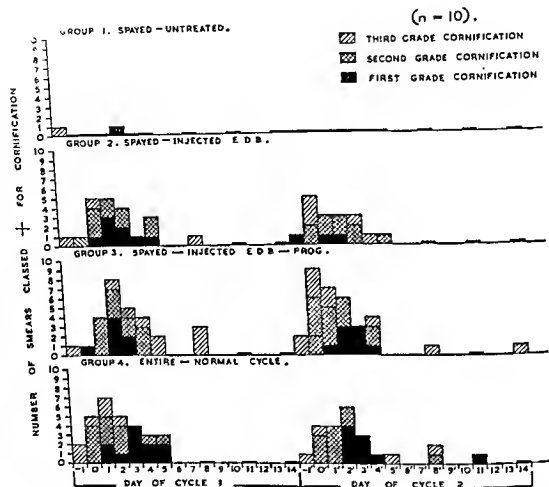


FIG. 6. Cyclic changes in the proportion of vaginal smears classed as positive for estrogenic activity. Grades of cornification are described and illustrated in Table II and Fig. 7. Group treatments and reference are as in Fig. 4. $n = 10$.

4. Practical Uses

The smear pattern has been used to reveal ovarian activity in anestrus. Changes characteristic of normal cycles have been observed and are almost certainly indicative of "silent" heats (110).

Opinions differ concerning the reliability of the smear for the determination of the stage of the cycle (21, 44, 88, 104, 112). Individual smears are of little value but a succession reveals a fairly clear-cut pattern (110, 125). Practical application is confined to the determination, with teasing, of the stage of estrus for artificial insemination (75).

V. ENDOCRINE CONTROL OF THE CYCLE

A. Cyclic Changes in Hormone Production

1. Anterior Pituitary Hormones

It is generally assumed that the initiation and subsequent control of estrous cycles at puberty and at the beginning of each breeding season

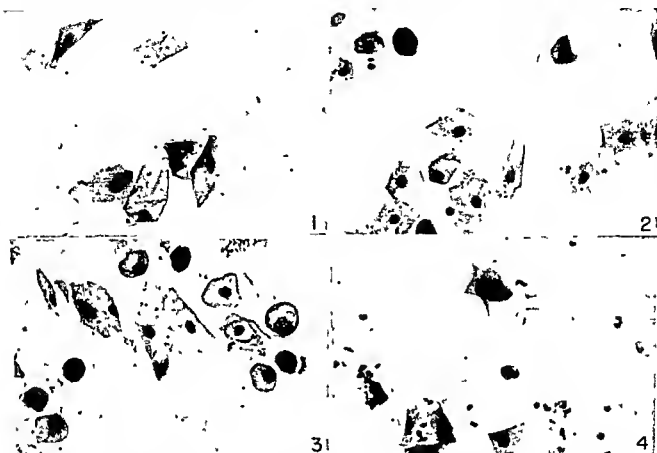


FIG. 7. Vaginal smears showing various degrees of cornification (see Table II). Those shown above and at bottom left would be classed as "positive"; that shown at bottom right as "negative". Magnification: $\times 280$.

Top left: Fully cornified smear (grade 1). Top right: Almost fully cornified smear (grade 2). Smear consists almost entirely of cornified and large squamous cells. Bottom left: Largely cornified smear (grade 3). Smear consists of cornified, squamous, and nucleated epithelial cells. Leucocytes are virtually absent. Bottom right: Slightly cornified smear (grade 4). Smear consists of cornified, squamous, and nucleated epithelial cells, plus large numbers of leucocytes. From Robinson and Moore (125).

is effected by quantitative or qualitative changes in the gonadotropin complex elaborated by the hypophysis. Photic and other neurogenic stimuli are involved.

Such seasonal fluctuations in pituitary activity—if any—are not reflected in the gonadotropin content of the gland. Indeed, Warwick (145) and Kammlade *et al.* (67) found the hypophysis of anestrus to

gonadotropin content at least as high as in the breeding season does not rule out the possibility of qualitative changes in the gonadotropin complex, nor does it necessarily indicate being produced and released into the blood stream.

... evidence for cyclic changes in pituitary gonadotropin content

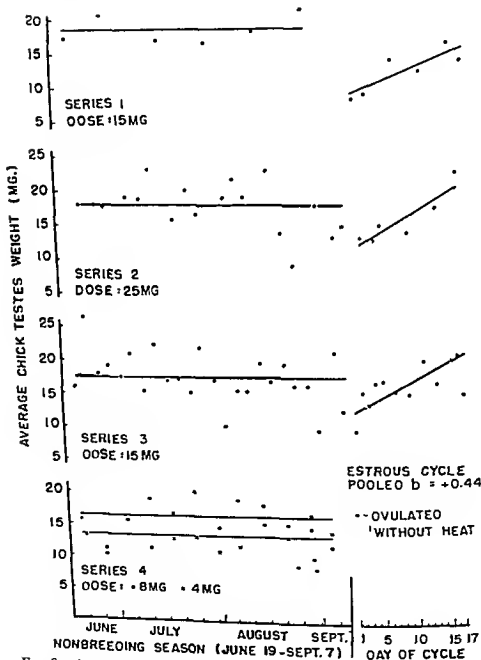


FIG. 8. Average levels of gonadotropic hormone in anterior pituitaries of sheep during the anestrus period and the estrus cycle. From Kammlade *et al.* (67).

within the cycle is more satisfactory Kammlade *et al* (67) observed a steady increase in the average potency of the sheep's pituitary from a low point at estrus to a maximum in late diestrus and proestrus. This was correlated with the size of the follicles (Figs 8 and 9). Cellular changes in the pituitary are not well marked and are limited to the degree of granulation which reaches a maximum early in diestrus. Application of the acidophil basophil classification of cell types is not practicable (144).

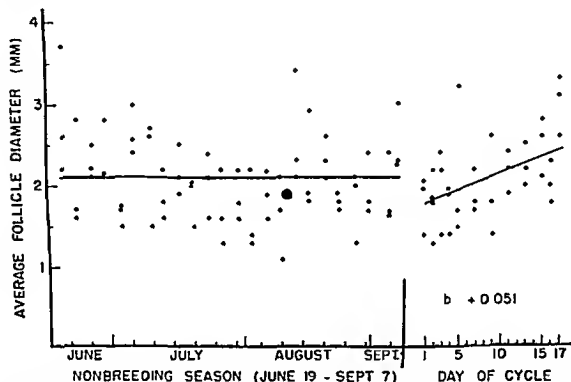


FIG 9 Average diameter of ovarian follicles during the anestrus period and the estrous cycle. The close parallel with pituitary gonadotropin levels (Fig 8) is apparent. From Kammlade *et al* (67).

There is evidence also of cyclic changes in the blood level of thyrotropic hormone (41), which is reported to be significantly higher at estrus than in diestrus. Thyroid activity obviously is an important factor in cyclic phenomena, but it is not clear how fundamental this is in the estrous cycle.

2 Gonadal Hormones

Evidence of cyclic changes in the production of ovarian hormones is conflicting. Brissett *et al* (8, 9) were unable to detect any difference between the amount of estrogen excreted daily in estrus and diestrus (1.1–2.4 µg estrone equivalent). None could be detected in anestrus. This led them to suggest that the production of ovarian estrogen is

the breeding season and that estrus is due to a fall in circulating progesterone.

Concerning the quantitative levels of progesterone is depending on the method of assay, of which there are two. The bio-assay method of Hooker and Forbes (64) yields results for blood from 4- to 20-fold higher than the chemical method of Edgar (35). On the assumption that both techniques are valid, the presumption is that the former measures total progestational activity in the blood, whereas the latter measures progesterone itself.

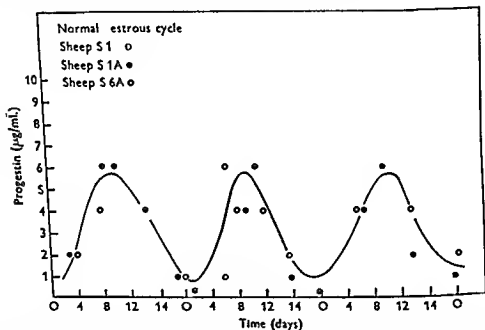


FIG. 10. Peripheral blood levels of progesterone as determined by the biological assay method of Hooker and Forbes (64) during the estrous cycle of the ewe. The cyclic changes parallel the development and regression of the corpus luteum. From Neher and Zarrow (96).

Neher and Zarrow (95, 96), using the bio-assay technique, found peripheral blood concentrations of cyclic ewes to increase from 0.3 to 2 µg. at estrus to 6 µg. in the mid-luteal phase (Fig. 10). Edgar (36) and Edgar and Ronaldson (37), on the other hand, have been unable to detect progesterone in peripheral blood of cyclic or pregnant ewes (sensitivity of test 0.1 µg. per ml.). Detectable but highly variable amounts were present in the ovarian vein draining an active ovary from the 3rd or 4th day. This reached a maximum (0.5 to 4.0 µg. per ml.) by the 7th to 9th day, remained high until the 16th, and fell suddenly on the 17th (Fig. 11). This finding is particularly interesting, as fatty degeneration of the corpus luteum normally commences about

the 14th day and appears to proceed rapidly thereafter (107). Failure to detect progesterone in the peripheral blood led Edgar to suggest that it was utilized as rapidly as it was secreted.

Replacement studies in spayed ewes have presented strong evidence of cyclic estrogen-progesterone production by the ovary and of physiological time-dose relationships. Alternate progesterone and estrogen influence is necessary for the maintenance of regular cyclic phenomena. The former must operate for a period of 12 to 14 days in relatively large quantities equivalent to daily injections in oil of 6 to 10 mg. and the lat-

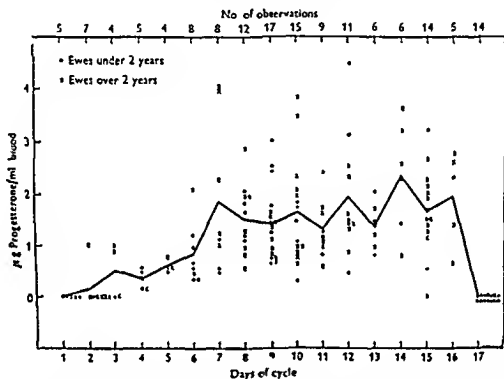


FIG. 11. Ovarian venous blood levels of progesterone as determined by the chemical method of Edgar (35) during the estrous cycle of the ewe. No progesterone can be detected in peripheral blood by this method at any stage of the cycle. From Edgar and Ronaldson (37).

ter for a short period of a day or so and in minute quantities equivalent to daily injections in oil of 20 to 25 µg. estradiol benzoate (118, 121, 126).

B. Control of Ovulation

It is generally accepted that ovulation is induced by the release of LH, but the mechanism of this release is obscure and varies from one type of animal to another. In spontaneously ovulating polyestrous animals it seems that ovulation and the ensuing cyclic phenomena are controlled by an ovarian-hypophyseal interaction. Pituitary FSH, with a relatively small amount of LH, induces follicle growth and maturation accompanied by estrogen production; circulating estrogen suppresses

FSH and stimulates LH; LH induces ovulation and corpus luteum formation; prolactin stimulates progesterone production; progesterone inhibits FSH and LH production and the cycle can only be repeated when the active life of the corpus ends. There is a wealth of experimental evidence to support this general concept, although there are differences of opinion concerning the nature of the hypophyseal gonadotropin complex and whether or not two discrete hormones are indeed produced. A major problem is the manner by which gonadal influence on the hypophysis is exercised, and about which there is considerable controversy. There appear to be but two alternatives for the sheep: (a) the circulating gonadal hormones influence the pituitary directly (humoral control, as is that of the gonads by the hypophysis), or (b) the control is mediated via a neural center, located presumably in the hypothalamus (neurohumoral control). Neural control of ovulation or indeed of any phase of the normal cycle may be of secondary importance. Nevertheless, uterine proprioceptors can exert a profound influence on pituitary function of the ewe. It has been shown that the ovarian follicular-luteal phase relationship can be severely upset by premature distention of the uterus; this appears to be a neural effect (91, 94). Furthermore, prolactin production in pregnancy is probably under neural control exercised via uterine proprioceptors. On the other hand, persistence of the corpus luteum—attributable to the influence of prolactin (92)—has been reported in cyclic ewes in which the uterus has been completely excised (150).

There is no direct evidence of the manner by which ovulation is controlled in the ewe, but a working hypothesis can be developed from the following experimental evidence. Ovulation may readily be induced in anestrus by the injection of gonadotropins, such as PMS, which are predominantly follicle-stimulating in action (18, 19, 40, 76, 115, 136). The efficiency of this reaction is improved if anterior pituitary (AP) gonadotropin, with a more balanced FSH-LH ratio, is used (10, 58, 93).

Luteinization generally follows such induced ovulation in a normal manner and, as overt estrus usually accompanies ovulation induced one cycle later, the corpus luteum appears functional. However, such treatment is not normally followed by a succession of cycles unless additional injections of gonadotropin are spaced one cycle apart (115). On the other hand, manipulation of the photic environment will induce a succession of cycles after a delay of some weeks (154, 155). The pituitary in anestrus has a gonadotropin content—of unknown qualitative nature—at least as high as in the breeding season (67, 145).

Whereas the injection of 1000 I.U. PMS in the follicular phase of a normal cycle will reliably induce multiple ovulation (up to 20 ova shed) it will rarely do so in anestrus (18, 113). Anterior pituitary gonadotropins are more reliable (58). Ovarian response to injected gonadotropin reaches a minimum in mid-anestrus, and some breeds with a deep anestrus may be quite refractory (113). Ovulation in a proportion of cases may follow either a single injection of estrogen in anestrus (57, 58) or cessation of a series of injections of progesterone (30, 31, 117).

Ovulation in a normal cycle is inhibited by daily injections of progesterone, and usually occurs some 2 or 3 days after cessation (33, 65, 98, 122).

The existence of an anestrus period, the ability to induce an ovulation by gonadotropin injection in anestrus, the inability of such a single injection to initiate a series of cycles, and the fact that a series of cycles can only be induced, after a conditioning period, by manipulation of the photic environment, all point to a pituitary inadequacy as being the cause of the anovulatory state of anestrus. This is at variance with the demonstration of a high gonadotropin content in anestrus. There are two possible explanations: (a) the nature of the gonadotropin complex in anestrus is abnormal, or (b) the mechanism which controls the release of either FSH or LH is not developed. Evidence of a high pituitary content of total gonadotropin and of the presence of follicles in the anestrus ovary suggests that release of LH may be the key to the problem.

Only the latter concept fits all the known facts, and suggests the following hypothesis. Control of the release of pituitary gonadotropin is effected by a center located, presumably, in the hypothalamus. Activation—and possibly deactivation—of this center is under the control of exteroceptive stimuli. Deactivation may be due in part to the development of a refractory condition to circulating gonadal hormones. In the almost fully reactive state, this center initiates a series of cycles by stimulating the release of gonadotropin, and is itself sensitive to the fluctuating levels of gonadal and possibly hypophyseal hormones. It thus acts as a center which regulates pituitary activity and hence the phenomena associated with the cycle. Toward the end of the breeding season this center becomes less efficient as the result either of its deactivation caused by exteroceptive stimuli, or to an increasing refractoriness to circulating levels of hormones, until finally it ceases to operate altogether, ovulations cease, and the ewe enters anestrus.

The relatively poorly developed center could exhibit some activity

in anestrus. For example, some stimulus, external or internal, could trigger the center to induce the release of gonadotropin (e.g., LH), with a consequent ovulation and "silent" estrus. A succession of cycles would not generally follow because the center is not sufficiently developed to react to the circulating levels of gonadal hormones. Injections of such hormones in large doses, however, may stimulate the rudimentary center to activity in some cases, again with a consequent "silent" estrus.

This hypothesis explains also the difficulty experienced in inducing multiple ovulations in anestrus. It is believed that the pituitary plays a significant role in such induced ovulations (58), and relative inefficiency of the LH release mechanism would account for the lack of multiple ovulations.

According to this hypothesis, control of ovulation in the ewe is by a neurohumoral mechanism operating through a hypothalamic center, the efficiency of operation of which fluctuates under the influence of photic and possibly other exteroceptive stimuli.

C. Control of Estrus

Early attempts by Hammond, Jr. *et al.* (58) to test the hypothesis that progesterone produced by a waning corpus luteum plays a role in estrous behavior were unsuccessful. The injection of 2 to 5 mg. progesterone up to 2 days before and 1 day after gonadotropin treatment failed to elicit an estrous response, although several ewes showed behavior characteristic of proestrus. In 1945, Cole *et al.* (18) reported heat in 39 of 48 anestrus ewes treated with 40 to 100 mg. testosterone propionate 1 to 3 days prior to PMS. Conception rates were low. These results were confirmed by Robinson (113), who found, on autopsy, that the androgen had inhibited or delayed ovulation. In some cases anovulatory estrus had occurred and follicles were grossly cystic and prematurely luteinized (Fig. 12).

The abnormal nature of the ovarian picture obtained with testosterone plus PMS seriously limited the value of these experimental results for the interpretation of normal endocrine interactions in overt estrus. The effect of the testosterone could have been to either (a) delay or inhibit ovulation, but not follicular growth, for a period long enough to permit a build up of sufficient estrogen to exceed the threshold needed to induce heat, or (b) condition a neural center to respond to follicular estrogen.

In 1952 there appeared three independent reports of the successful use of progesterone prior to PMS for the induction of ovulation accompanied by estrus in anestrus ewes (26, 30, 116). These reports were

later confirmed and extended (23, 31, 117, 119, 121). Subsequent work with both spayed and entire ewes has considerably clarified our understanding of the endocrine control of estrus.

Early reports on the use of estrogen to induce heat in spayed or anestrus ewes had shown wide discrepancies in the effective doses (19, 40, 84, 106). Nevertheless it was still generally accepted that this was the only hormone involved. The necessity for progesterone prior to estrogen for the regular induction of normal cyclic estrous behavior in spayed ewes was first shown in 1954 (118). Twelve ewes, of which 6 were hysterectomized, were injected with estradiol benzoate (EDB), first at weekly and later at 2-week intervals, at dose levels of either



FIG. 12 Ovarian slices from anestrus ewes pretreated with 35 mg. testosterone propionate prior to the injection of 800 I.U. serum gonadotropin (PMS). Anovulatory estrus associated with grossly cystic follicles is a characteristic of this treatment. From Robinson (113).

5 or 2 mg. Figure 13 shows the pattern of behavior. Eleven of the 12 ewes were served after the first injection. The number of ewes served declined with successive treatments until a completely refractory condition developed.

The injection of 75 mg. progesterone, spread over a period of 3 days and commencing 4 days before the injection of EDB, completely removed this refractory condition. The ewes were then divided into two groups each of 6 ewes of which 3 were hysterectomized. Injections of EDB at fortnightly intervals were repeated 3 times at each of 5 dose levels ranging from 5 mg. down to 8 μ g. Ewes of one group received EDB only, whereas the others were "primed" with progesterone before each injection. The ability of any ewe to experience a succession of heat periods in response to these injections of EDB was entirely dependent on her receipt of progesterone prior to each injection of estro-

gen, the critical dose level of which was about 20 μ g. Presence or absence of the uterus had no demonstrable effect.

Further quantitative work (88, 89, 120, 126), using a flock of 72 spayed standardized crossbred ewes, has yielded results and conclusions which may be summarized as follows.

The spayed crossbred ewe, when primed with progesterone, is extremely sensitive to estrogen; this sensitivity increases linearly with increase in the log duration of progesterone influence. The unprimed ewe

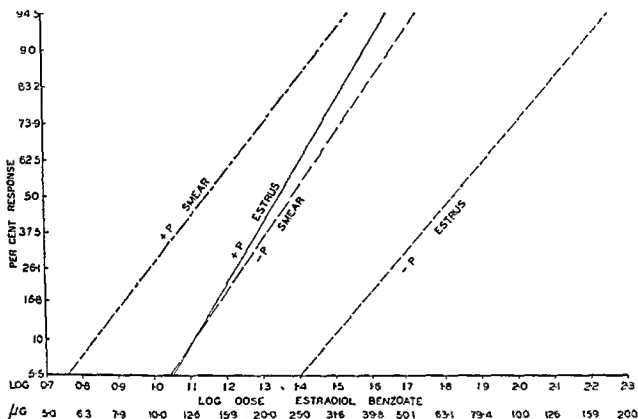


FIG. 14. Dose-response lines of EDB for estrous behavior and for vaginal smear, determined in spayed ewes, when injected alone or preceded by 75 mg. progesterone (6 injections each of 12.5 mg. twice daily, commencing 5 days before EDB). — P = EDB alone; + P = EDB preceded by progesterone. From Robinson (120).

is much less sensitive (Table III, Figs. 14, 15, and 16). Furthermore, the reliability of response to estrogen, as indicated by the induction of successive heats (Fig. 13) and by the slope of the dose-response line, is significantly increased by progesterone pretreatment (Fig. 14), and the time of onset of estrus is significantly advanced (Fig. 17).

These effects of progesterone are not due to an "estrogen sparing" effect. The reaction pattern is altered. For example, when estrogen is injected alone there is no evidence of a dose-response effect on the time of onset of heat. Progesterone pretreatment results not only in an earlier

TABLE III

ESTIMATES OF ED_{50} , ED_{90} , AND ED_{99} OF ESTRADIOL BENZOATE (EDB) FOR ESTRUS WHEN INJECTED FOLLOWING VARIOUS PERIODS OF PRETREATMENT WITH EFFECTIVE DOSES OF PROGESTERONE (126)

Duration of progesterone pretreatment (days)	Estimates of EDB (μ g.) with 95% fiducial limits								
	ED_{50}			ED_{90}			ED_{99}		
	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper
3	17.0	18.7	20.5	24.5	27.0	30.7	28.6	32.1	38.1
6	14.8	15.7	16.6	20.9	22.6	25.3	24.3	26.9	31.5
12	11.9	13.1	14.5	17.0	18.9	22.0	19.8	22.5	27.2

onset of estrus, but also in a significant relationship between the dose level of EDB and the time of such onset (Fig. 18).

When progesterone pretreatment is spread over 12 days—the approximate duration of influence in a normal cycle—there is very little discrepancy between the quantities of estrogen required to produce both

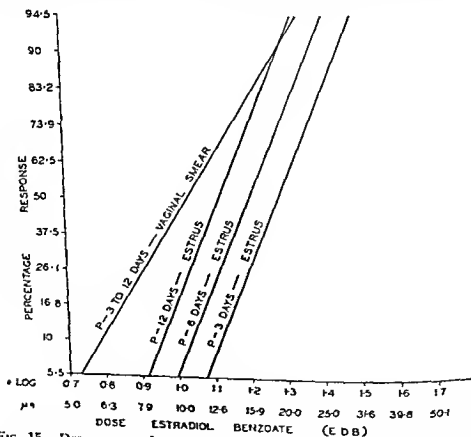


FIG. 15. Dose-response lines of EDB for estrus and for vaginal smear, determined in spayed ewes, when preceded by progesterone (P) injected for 3, 6, or 12 days. From Robinson *et al.* (126).

TABLE IV
ESTIMATES OF ED_{50} , ED_{90} , AND ED_{99} OF ESTRADIOL BENZOATE (EDB) FOR VAGINAL
ESTROUS CHANGES (126)

Fiducial limits (%)	Estimates of EDB ($\mu\text{g.}$) with 95 and 99% fiducial limits							
	ED_{50}			ED_{90}			ED_{99}	
	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Upper
95		10.7			18.6		24.1	
99	8.4		12.1	10.8		21.9	20.8	32.7
	7.3		12.5	10.4		24.0	20.1	39.3

the psychic and the vaginal changes characteristic of estrus (Tables III and IV, Fig. 15). The estimates of ED_{50} , EDB, with their associated 95% fiducial limits are 11.9-13.1-14.5 $\mu\text{g.}$ and 8.4-10.7-12.1 $\mu\text{g.}$, respectively.

The effective daily dose of progesterone lies between the extreme limits of 3 and 24 mg. per day, with the optimum between 6 and 12 mg.

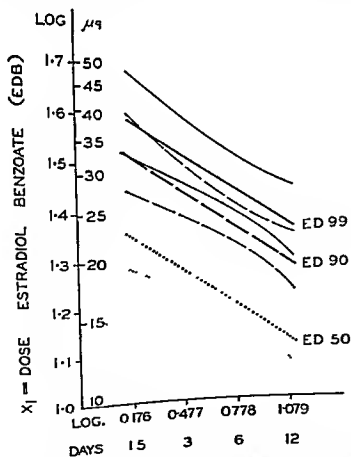


FIG. 16. Graphs of ED_{50} , ED_{90} , and ED_{99} lines of EDB for estrous behavior, with their 95% fiducial bands. The narrow bands indicate the reliability of the estimates and the precision with which the response to estrogen of the spayed crossbred ewe may be estimated following progesterone pretreatment. From Robinson *et al.* (126).

(126). The maximum expression of the progesterone-estrogen interaction is dependent on highly critical time-dose relationships. Simultaneous injection of the two hormones in physiological doses (12 mg. per day and 16 to 25 μ g. per day, respectively) gives no estrous response. Maximal responses are obtained when EDB follows 24 to 48 hours after the final injection of progesterone, after which there is a significant regression of response on time (Table V, Fig. 19).

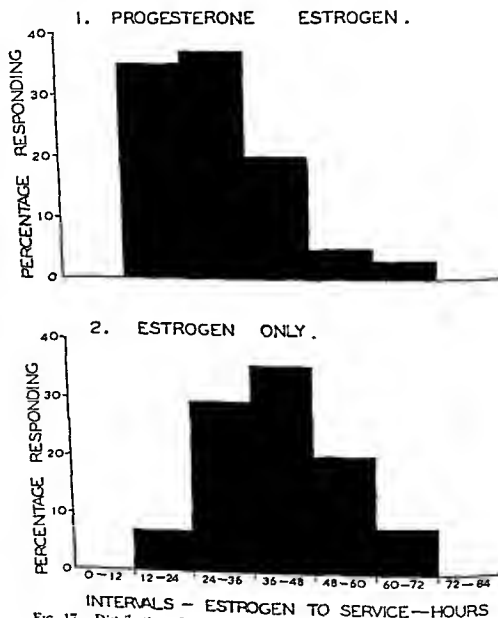


FIG. 17. Distribution of time of onset of estrus in spayed ewes when EDB is injected alone or following progesterone pretreatment. The advance in time of onset of estrus is highly significant ($P < 0.001$). From Robinson (120).

Related studies on intact anestrus ewes treated with various time-dose relationships of progesterone, estrogen, and gonadotropin give a good indication of the quantity of estrogen produced by a developing follicle. If such ewes are pretreated with progesterone for 3, 6, or 12 days prior to the injection of 1000 I.U. PMS, approximately 50, 90, and 99%, respectively, of those which ovulate may be expected to accept the ram. Reference to Table III and Figs. 15 and 16 clearly indicates

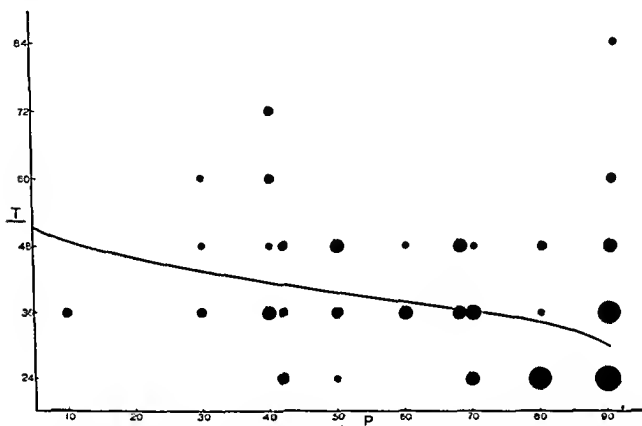


FIG. 18. Regression of T (time in hour to onset of estrus) on P (percentage of ewes served as a result of treatment) of spayed ewes injected with EDB after pretreatment with progesterone. The slope of the regression is significant ($P < 0.05$), indicating earlier onset of estrus with increasing proportions of ewes served, and hence of increasing dose levels of EDB. No such relationship can be demonstrated when EDB is injected without prior progesterone treatment. The area of each \bullet is proportional to the number of observations. From Robinson (120).

that this corresponds to a production by the developing follicle of a quantity of estrogen approximately equivalent in physiological activity to a single injection in oil of 20 to 25 μ g. EDB (121, 126). Such a quantity of estrogen is inadequate to induce heat in any but a very small proportion of ewes which have not previously been sensitized by progesterone.

There is some evidence that the amount of estrogen produced by the maturing follicle is dependent on the amount and nature of the circulating gonadotropin. Emmens *et al.* (38) have thrown considerable

doubt on the generally accepted differences between pregnancy gonadotropins of equine (PMS) and human (HCG) origin, as tested by uterine weight responses in rodents. When tested in the normal cyclic ewe in which ovulation is inhibited by progesterone injections (see below), and subsequently stimulated by simultaneous release of progesterone inhibition and the injection of 100 or 600 I.U. gonadotropin, the action of HCG (Parke Davis) is indistinguishable from that of PMS (127).

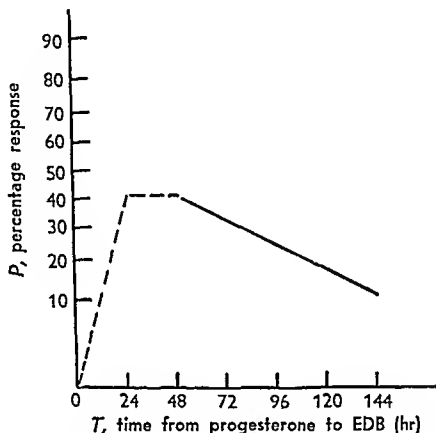


FIG. 19. Percentage of spayed ewes exhibiting estrous behavior (P) plotted against interval (T) between final injection of progesterone and injection of EDB. Maximum responses are shown when EDB follows 24 to 48 hours after progesterone. Thereafter, simple linear regression of P on T is shown over the range + 48 to + 144 hours. From Moore and Robinson (90).

On the other hand, when tested in anestrus ewes, some interesting differences between the action of the two gonadotropins become apparent (Table VI). Of 72 ewes which received 250, 500 or 1000 I.U. PMS or HCG after 12 daily injections of progesterone, 66 ovulated and 42 were served—29 of 33 which ovulated after PMS, as compared with 13 of 33 which did so after HCG ($P < 0.01$). Furthermore, there appeared to be a dose/estrous-response effect with PMS. This was particularly apparent in the ewes primed at 4 daily intervals and appeared unrelated to the number of ovulations (124).

There are two possible explanations, namely, (a) the time relation-

ships of follicle development, estrogen production, and ovulation relative to the progesterone status of the animal are influenced by the amount and nature of the gonadotropin used, or (b) the amount of estrogen produced is affected. The animals were killed 4 to 6 days after gonadotropin injection and there was nothing in the appearance of the ovaries and the corpora lutea to support the former possibility. The second possibility seems the more likely, therefore, and it appears that a dose of 1000 I.U. PMS in the anestrous ewe induces the production of a quantity of estrogen roughly equivalent to a single injection of about 20-25 µg. estradiol benzoate. Such a dose would be expected to yield estrus in all ewes primed daily for 12 days with progesterone, and in about 50% of ewes given 3 priming injections spaced 4 days apart (124). Smaller doses, while capable of inducing ovulation, apparently produce marginal quantities of estrogen. Chorionic gonadotropin, while equally effective in inducing ovulation, appears much less efficient in stimulating the production of ovarian estrogen.

TABLE VI

EFFECT OF EQUINE GONADOTROPIN (PMS) AND CHORIONIC GONADOTROPIN (HCG) ON OVULATION AND ESTRUS IN THE PROGESTERONE-PRIMED ANESTROUS EWE (124)^a

		Gonadotropin and dose						Total ewes
		PMS (IU)			HCG (IU)			
Progesterone	Ewes	250	500	1000	250	500	1000	
Daily	Ovulated	11	10	12	12	11	10	66
	Served	8	9	12	6	5	2	42
Every 4 days	Ovulated	12	12	12	11	10	9	66
	Served	0	0	6	0	0	0	6

^a Progesterone (intramuscular) in oil at dose level of 8.4 mg./day, injected daily for 12 days or as 3 injections 4 days apart. PMS or HCG (subcutaneous) in distilled water injected on the 13th day. $n = 12$.

It is possible, therefore, to explain fully the phenomenon of "silent" heat. Functional failure of a number of cyclic processes would result in ovulation without estrus. Absence of a corpus luteum from a previous cycle, subfunction due to a prolactin deficiency, or premature atrophy would each result in failure of the progesterone-conditioning mechanism. Alternatively, ovulation induced by a marginal amount of gonadotropin—as might be expected early or late in the breeding season—could be accompanied by submarginal production of estrogen. Each type of failure is probably important at different stages of the breeding season.

The mechanism of the progesterone-conditioning effect is obscure. The uterus does not appear to be involved, so it is likely that the role of progesterone is to increase the sensitivity to estrogen of a center presumably located in the central nervous system. This appears to be a gradual process in which the duration of stimulation rather than the absolute amount of progesterone—within rather wide limits—is the important factor. From the work of Santolucito *et al* (129) it appears that this center may be located in the hypothalamus. Electrolytic lesions in the ventral hypothalamus, with a common area just above the median eminence, abolished heat in cyclic ewes without causing any apparent change in the cyclic release of pituitary gonadotropin.

D Control of Changes in the Reproductive Tract

Cyclic changes in the reproductive tract are under the dual control of estrogen and progesterone. Injection of estrogen in spayed or anestrus ewes, followed by progesterone, induces the characteristic histological changes first of estrus, then of met- and diestrus. The height of the cervical epithelium is increased by estrogen and this is maintained by progesterone (11).

The interaction of estrogen and progesterone on the vaginal smear has been studied recently (89, 90, 125). It appears that desquamation and cornification of the vaginal epithelium is under estrogenic control. The normal pattern of leucocyte invasion and subsequent disappearance appears dependent on an estrogen-progesterone interaction, since more or less complete disappearance of leucocytes on the day of estrus occurs only when the ewe has been under the influence of progesterone prior to estrogen (Fig 5). Progesterone is involved also in the normal thickening of the mucus in metestrus (Fig 4) and, with estrogen, in the production of mucus showing the phenomenon of "crystallization," characteristic of estrus (79).

The maintenance of a constant state of vaginal sensitivity to recurring injections of estrogen is dependent on alternate progesterone-estrogen influence. A series of six trials was conducted, each using 72 spayed ewes, in which EDB (8, 12, 18 μ g) was injected at intervals of 16 days. Half the ewes received progesterone at the rate of 10 mg per day for 12 days between each injection. The vaginal response to EDB of these ewes did not vary significantly from trial to trial, while that of the remainder showed a highly significant decline from a hyper- to hyposensitive state, clearly suggesting the development of a refractory condition (Fig 20).

It appears, therefore, that in addition to its role in maintaining a

constant and highly sensitive state of psychic reactivity to physiological amounts of estrogen, progesterone is involved also in the maintenance of a uniform vaginal sensitivity.

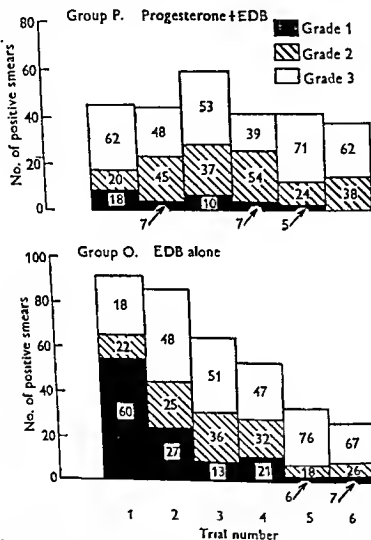


FIG. 20. Distribution of smears of various grades of cornification (Table II) in successive trials, when EDB is injected into spayed ewes at 16-day intervals, with or without progesterone pretreatment. Enclosed numerals refer to percentage of each grade of cornified smear in each trial. Maintenance of a uniform vaginal sensitivity is dependent on progesterone influence prior to each injection of estrogen. From Moore and Robinson (89).

VI. ARTIFICIAL CONTROL OF THE CYCLE

A. Economic Significance

The extended anestrus of many breeds of sheep presents an economic problem, a completely satisfactory solution of which has evaded intensive investigation for 25 years.

The number of ovulations at each estrus constitutes an important limiting factor to fertility. This may be effectively removed by the injection of gonadotropin in late diestrus. Field trials have resulted in significant increases in the number of lambs born.

Artificial insemination of the ewe is not widely practiced in western countries because of the high labor costs involved in the handling operations, the unreliability of prediction of the number of ewes in heat each day, and the general experience of unsatisfactory conception rates following dilution and storage of ram semen. Consequently, attention has been turned toward the synchronization of estrus and ovulation by controlling the length of diestrus by the use of progesterone.

The literature to 1951 has been reviewed (115). A number of significant advances have been made since then; a summary of the present situation follows.

B. Control in Anestrus

Ovulation in anestrus may be induced readily by the injection of 800 to 1000 I.U. PMS. One or 2, and rarely 3, ova are shed. If preceded by 6 to 10 mg. progesterone daily for 6 to 12 days, such gonadotropin-induced ovulations are generally accompanied by estrus (23, 26, 30, 31, 43, 72, 73, 116, 117, 121). Injection of 20 mg. progesterone every other day is also effective.

Equine gonadotropin should be injected 24 to 48 hours after the final injection of progesterone. Heat may occur within 24 hours (75) but ovulation does not occur until about 48 hours after the injection of PMS (124). The degree of fertility appears to be related to the stage of anestrus, and the relative fertility of the ram is almost certainly involved. Treatment in mid-anestrus commonly results in a conception rate of less than 50% (119, 121), whereas normal fertility has been reported following treatment just prior to the start of the normal breeding season (72, 73).

Treatment in mid-anestrus usually results in only a single cycle, whereas in late anestrus a series running into the normal breeding season ensues. Therefore, except in special cases, e.g., for stud use, the practical use of progesterone plus PMS is limited at present to the advancement of the breeding season by commencing treatment 6 weeks or so before the normal onset of breeding activity (72).

C. Control in the Breeding Season

1. Number of Ovulations

Work initiated in the U.S.S.R. showed that the number of ovulations could be increased by the injection of PMS or AP in the early follicular

phase of the cycle (77, 78, 157, 158, 159, 160, 161). This finding has been amply confirmed (15, 24, 58, 74, 93, 114). A single injection of PMS on the 11th to 13th day of the cycle is effective (135, 137, 140, 141, 142), and a dose-response relationship has been established (Table VII).

TABLE VII
OVULATIONS AND ATTACHMENTS OF FERTILIZED OVA IN RELATION TO THE DOSE OF PMS INJECTED IN THE FOLLICULAR PHASE OF THE CYCLE (114)

Treatment (IU)	No. of ewes	Ovulations	Attachments
500	15	3.9 (range 2-7)	3.1 (range 0-7)
1000	20	10.3 (range 4-29)	3.75 (range 0-10)
2000	5	18.8 (range 8-33)	4.4 (range 0-13)
All treatments	40	9.0	3.6

The wide range in the number of ovulations, apparent from Table VII, does not necessarily constitute a serious objection to the practical use of this technique. Within the limits of 2 to 9 ovulations, as observed following the injection of 500 I.U. PMS, ova are highly fertile. Subsequent embryonic mortality, usually within the first 21 days, reduces the number surviving to the maximum number characteristic of the breed (Fig. 21). Judicious use of PMS, therefore, enables ewes to express their maximum potential fertility (114).

Field use of this technique involves the use of teaser rams. Every 3rd day teased ewes are drafted. Eleven days later each is injected with 500 to 750 I.U. PMS and joined to fertile rams. Such treatment has been reported to raise lambing percentages by about 30% (42, 99, 140, 142).

2. Time of Ovulation

Daily injection of 10 mg. progesterone effectively inhibits ovulation in cyclic ewes (33, 65, 98, 122); if such treatment is continued for 16 days, estrus and ovulation can be fairly effectively brought into line in all ewes treated. From 50 to 70% of ewes can be expected in estrus 3 days after cessation of injections. The precision of time of onset of estrus and of ovulation can be considerably increased by the injection of 500 I.U. PMS the day following the final progesterone treatment (122).

Attempts to reduce the number of injections of progesterone have not been satisfactory. Practical application demands uniform time of withdrawal of progesterone influence, and this can only be obtained effectively when small, fairly rapidly absorbed injections are given. Hence, while large doses in oil or moderate doses in suspensions or emulsions given at intervals of up to 4 days effectively suppress ovula-

tion, the time of release of the inhibition is not predictable (124). Eight injections each of 20 or 33.4 mg. progesterone in oil every other day effectively suppress estrus, but time relationships for estrus and ovulation following cessation of such treatment differ slightly from those following daily treatment, and conception rates to artificial insemination are significantly reduced (123).

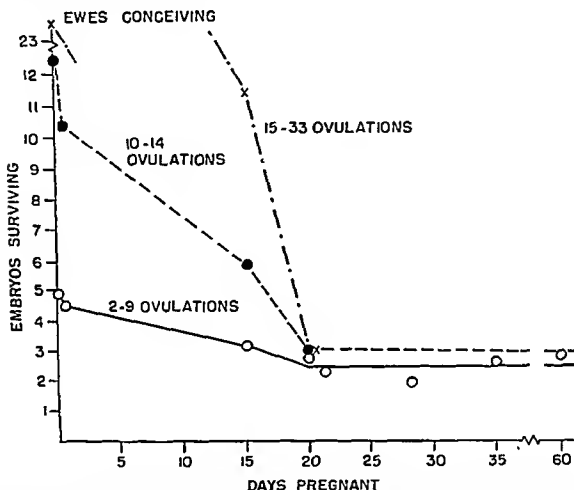


FIG. 21. Survival of embryos in the multiple-ovulated ewe relative to the number of ova shed. This refers only to those ewes which conceive. Total failure is common when the number of ovulation exceeds 15. A dose of 500 I.U. equine gonadotropin (PMS) can be expected to yield between 2 and 9 ovulations. Early embryonic mortality usually reduces the number surviving to between 1 and 4. From Robinson (114).

When PMS is injected the day following cessation of 16 daily injections of progesterone, ewes may be teased within 24 hours. If they are artificially inseminated immediately, poor conception rates may be expected (75), as ovulation does not usually occur within 48 hours or so of this injection (124).

Potential fertility following such controlled ovulation generally appears unimpaired and results have been satisfactory using relatively small numbers and either natural service or artificial insemination (33,

98, 122). Application of the technique to large-scale, controlled artificial insemination has been disappointing, with conception rates as low as 30% (123). This is probably due largely to factors operating outside the ewe, although critical timing relationships between injections and ovulations may be important when large numbers are treated daily. The margin between success and failure in artificial insemination of the ewe is extremely narrow and the additive effects of many factors, such as the necessity to use whatever semen is available on the day, regardless of its quality, the need for speed and consequent lack of attention to fine detail, and fatigue of the operator, must each contribute seemingly disproportionate amounts to the failure rate.

D. Conclusions

Our understanding of the factors which control the estrous cycle of the ewe has increased greatly in the last decade. The practical application of this knowledge has been a little disappointing, but the barrier between principle and practice is not insurmountable. The key to the control of breeding phenomena is the use of progesterone. The development of slowly, uniformly absorbing preparations, coupled perhaps with the replacement of PMS by a cheaper gonadotropin, will enable the phenomena of estrus and ovulation to be brought under complete and economical control. This achievement will be full justification for the enormous effort which has followed the original induction of ovulation in the anestrus ewe by Cole and Miller (20) some 25 years ago.

Part II: The Doe

VII. INTRODUCTION

The characteristics of the cycle of the doe appear essentially similar to those of the ewe. She is a seasonally polyestrous animal that reaches her peak of reproductive activity in the autumn (4, 5). The characteristic number of annual cycles exhibited by the various types and breeds appears to be related, as in the ewe, to the severity of the environment in which they evolved. Rhythmic breeding activity is under photoperiodic control (12, 34).

Despite the practical problem posed by the restricted breeding season, the estrous phenomena have been relatively little studied. The most comprehensive account available is that of Phillips *et al.* (102).

VIII. CHARACTERISTICS OF THE CYCLE

Kids born in the spring exhibit their first estrous cycle in the late autumn of the same year (70).

The length of the cycle is generally given as 21 days, but it is highly variable. Figures based on accurate measurements are 19.4 ± 0.5 days, with a range of 12 to 24 (105) and 17.8 ± 0.36 days, with a range of 6 to 24 (103). Estimates of mean cycle length are biased by the high proportion of extremely short cycles. Thus Phillips *et al* (103) observed a very strong mode at 20 days, 72% of their observations fell between 15 to 24 days. Hence the normal length of the cycle may be regarded as 20 days.

The duration of estrus (40 hours) is rather longer than in the ewe (105). Ovulation is spontaneous and occurs some 30 to 36 hours after the onset of heat (61).

The number of ova shed is rather greater than in the sheep. The usual number of young born is 2, 1 or 3 are common, 4 or 5 are rare (5).

The development of the corpus luteum is similar to that in the ewe. It reaches its maximum size, and presumably physiological activity, in mid cycle and degenerative changes appear after the 15th day (6).

The cyclic changes in the uterine mucosa and vagina have been described by Hamilton and Harrison (52). Changes are generally similar to those seen in the sheep. The most pronounced changes are seen in the vaginal epithelium, while evidence of secretory activity parallels the development and degeneration of the corpus luteum.

Although estrus with ovulation has been reported when anestrus does are injected with PMS (1, 103), Dauzier *et al* (23) report "silent" heats, and an estrous ovulation response only when progesterone precedes gonadotropin injection in anestrus. It appears almost certain, therefore, that a progesterone-estrogen relationship exists for the doe as for the ewe, but the temporal and quantitative relationships may be somewhat different.

REFERENCES

- 1 Ajello, P., and Lombardo, N., *Zootec e vet* 10, 2 (1955)
- 2 Allden, W. G., *J. Dept. Agr. S. Australia* 69, 337 (1956)
- 3 Anderson, J., *J. Agr. Sci.* 28, 64 (1938)
- 4 Asdell, S. A., *J. Agr. Sci.* 16, 632 (1926)
- 5 Asdell, S. A., *Patterns of Mammalian Reproduction*. Comstock, Ithaca, New York, 1946
- 6 Assheton, R., *Quart. J. Microscop. Sci.* 41, 205 (1898)
- 7 Averill, R. L. W., in *Studies on Fertility* (R. G. Harrison, ed.), Vol. VII, p. 139. C. C. Thomas, Springfield, Illinois, 1955
- 8 Bassett, E. G., and Sewell, O. K., *Nature* 167, 356 (1951)
- 9 Bassett, E. G., Sewell, O. K., and White, E. P., *New Zealand J. Sci. Technol.* A36, 437 (1955)
- 10 Bell, T. D., Casida, L. E., Bolstedt, G., and Darlow, A. E., *J. Agr. Research* 62, 019 (1941)

11. Bell, T. D., Casida, L. E., and Darlow, A. E., *Endocrinology* 28, 441 (1941).
12. Bisonette, T. H., *Physiol. Zool.* 14, 379 (1941).
13. Briggs, H. M., Darlow, A. E., Hawkins, L. E., Wilham, O. S., and Hauser, E. R., *Oklahoma Agr. Expt. Sta. Bull. No. 255* (1942).
14. Casida, L. E., and McKenzie, F. F., *J. Animal Sci.* 4, 24 (1932).
15. Casida, L. E., Warwick, E. J., and Meyer, R. K., *J. Animal Sci.* 3, 22 (1944).
16. Clark, R. T., *Anat. Record* 60, 125 (1934).
17. Clark, R. T., *Anat. Record* 60, 135 (1934).
18. Cole, H. H., Hart, C. H., and Miller, R. F., *Endocrinology* 36, 370 (1945).
19. Cole, H. H., and Miller, R. F., *Am. J. Physiol.* 104, 165 (1933).
20. Cole, H. H., and Miller, R. F., *Anat. Record* 58, Suppl., 56 (1934).
21. Cole, H. H., and Miller, R. F., *Am. J. Anat.* 57, 39 (1935).
22. Dautzier, L., *Compt. rend. soc. biol.* 147, 1556 (1953).
23. Dautzier, L., Ortavant, R., Thibault, C., and Wintenberger, S., *Ann. endocrinol. (Paris)*, 14, 553 (1953).
24. Dautzier, L., Thibault, C., and Wintenberger, S., *Ann. Zootech. Paris* 2, 189 (1953).
25. Dautzier, L., and Wintenberger, S., *Ann. Zootech. Paris* 1, 13 (1952).
26. Dautzier, L., and Wintenberger, S., *Ann. Zootech. Paris* 1, 49 (1952).
27. Dautzier, L., and Wintenberger, S., *Compt. rend. soc. biol.* 146, 67 (1952).
28. Dautzier, L., and Wintenberger, S., *Compt. rend. soc. biol.* 146, 660, 663 (1952).
29. Dautzier, L., and Wintenberger, S., *Proc. 2nd Intern. Congr. Physiol. and Pathol. Animal Reproduction and Artificial Insemination Copenhagen* p. 113 (1952).
30. Dutt, R. H., *J. Animal Sci.* 11, 792 (1952).
31. Dutt, R. H., *J. Animal Sci.* 12, 515 (1953).
32. Dutt, R. H., and Bush, L. F., *J. Animal Sci.* 14, 885 (1955).
33. Dutt, R. H., and Casida, L. E., *Endocrinology* 43, 208 (1948).
34. Eaton, O. N., and Simmons, V. L., *U. S. Dept. Agr. Circ. No. 993*, 16 pp. (1953).
35. Edgar, D. G., *Biochem. J.* 54, 50 (1953).
36. Edgar, D. C., *Nature* 173, 639 (1954).
37. Edgar, D. G., and Ronaldson, J. W., *J. Endocrinol.* 16, 378 (1958).
38. Emmens, C. W., Claringbold, P. J., and Lamond, D., *Nature* 180, 38 (1957).
39. El-Sheikh, A. S., Hulet, C. V., Pope, A. L., and Casida, L. E., *J. Animal Sci.* 14, 919 (1955).
40. Frank, A. H., and Appleby, A., *J. Animal Sci.* 2, 251 (1943).
41. Ghanem, Y. S., and Soliman, F. A., *Brit. Vet. J.* 112, 462 (1956).
42. Gordon, I., *Agr. Rev.* 3, 20 (1957).
43. Gordon, I., *Agr. Rev.* 3, 12 (1958).
44. Grant, R., *Trans. Roy. Soc. Edinburgh* 58, 1 (1934).
45. Green, W. W., and Winters, L. M., *Anat. Record* 61, 457 (1935).
46. Green, W. W., and Winters, L. M., *Minn. Univ. Agr. Expt. Sta. Tech. Bull. No. 169* (1945).
47. Hadek, R., *Nature* 171, 750 (1953).
48. Hadek, R., *Nature* 171, 976 (1953).
49. Hadek, R., *Vet. Record* 66, 632 (1954).
50. Hadek, R., *Anat. Record* 121, 187 (1953).
51. Hafez, E. S. E., *J. Agr. Sci.* 42, 189 (1952).

- 52 Hamilton, W J, and Harrison, R J, *J Anat* 85, 316 (1951)
- 53 Hammond, J, *J Agr Sci* 6, 263 (1921).
- 54 Hammond, J, "Farm Animals," Edward Arnold, London, 1952
- 55 Hammond, J, *Biol Rcls Cambridge Phil Soc* 22, 195 (1947)
- 56 Hammond, J, Jr, *J Agr Sci* 34, 96 (1944)
- 57 Hammond, J, Jr, *J Endocrinol* 4, 169 (1945)
- 58 Hammond, J, Jr, Hammond, J, and Parkes, A S, *J Agr Sci* 32, 308 (1942)
- 59 Hansel, W, Asdell, S A, and Roberts, S J, *Am J Vet Resorchi* 10, 221 (1949)
- 60 Hart, D S, *J Agr Sci* 40, 143 (1950)
- 61 Harrison, R J, *J Anat* 80, 160 (1948)
- 62 Hawkins, L E, and Darlow, A E, *Proc Am Soc Animal Production* 26, 274 (1933)
- 63 Heape, W, *Quart J Microscop Sci* 44, 1 (1900)
- 64 Hooker, C W, and Forbes, T R, *Endocrinology* 41, 158 (1947)
- 65 Hunter, G L, *J Endocrinol* 10, Proc, viii-xiv (1954)
- 66 Inkster, I J, *Sheepfarming Ann (New Zealand)* p 163 (1951)
- 67 Kammlade, W G, Welch, J A, Nalbandov, A V, and Norton, H W, *J Animal Sci* 11, 646 (1952)
- 68 Kelley, R B, Australia, *Commonwealth Sci Ind Research Organization Bull No* 205 (1946)
- 69 Kelley, R B, and Shaw, H E B, Australia, *Commonwealth Sci Ind Research Organization Bull No* 166 (1943)
- 70 Kiesling, A, *Zuchtungskunde* B27, 141 (1923)
- 71 Kupfer, M, *Rept Vet Resorchi S Africa* 13, 14, 1209 (1928)
- 72 Lambourne, L J, *New Zealand J Sci Technol Agr* 37, 187 (1955)
- 73 Lambourne, L J, *Proc Ruakura Farmers Conf New Zealand* p 18 (1956)
- 74 Laplaud, M, and Thibault, C, *Compt rend acad agr (France)* 33, 516 (1947)
- 75 Lloyd Davies, H, and Dun, R B, *Australia Vet J* 33, 92 (1957)
- 76 Lognova, N V, *Trans All Union Soc Resorchi Inst* 10, 91 (1939)
- 77 Lognova, N V, and Lopyrin, A I, *Problemy Zhivotnovodstva No* 10, 114 (1938)
- 78 Lopyrin, A I, "Multioctation of Sheep" Moscow, 1938
- 79 McDonald, M F, and Raeside, J I, *Nature* 178, 1472 (1956)
- 80 McKenzie, F F, Allen, E, Guthrie, M J, Warbritton, V, Terrill, C E, Casida, L E, Nahm, L K, and Kennedy, J W, *Proc Am Soc Animal Production* 26, 278 (1933)
- 81 McKenzie, F F, and Phillips, R W, *Proc Am Soc Animal Production* 23, 138 (1930)
- 82 McKenzie, F F, and Phillips, R W, *Repts Univ Missouri* June 1932
- 83 McKenzie, F F, and Terrill, C E, *Anat Record* 61, Suppl, 58 (1935)
- 84 McKenzie, F F, and Terrill, C E, *Missouri Univ Agr Expt Sta Research Bull No* 264 (1937)
- 85 Marshall, F H A, *Phil Trans Roy Soc London* B196, 47 (1903)
- 86 Marshall, F H A, "The Physiology of Reproduction," 2nd ed Longmans, Green, London, 1922
- 87 Marshall, F R, and Potts, G G, *U S Dept Agr Bull No* 996 (1924)
- 88 Mihaila, M, *Ann Inst Natl Zootech Roumanie* 5, 190 (1936).
- 89 Moore, N W, and Robinson, T J, *J Endocrinol* 14, 297 (1957)

90. Moore, N. W., and Robinson, T. J., *J. Endocrinol.* **15**, 360 (1957).
91. Moore, W. W., and Nalbandov, A. V., *Endocrinology* **53**, 1 (1953).
92. Moore, W. W., and Nalbandov, A. V., *J. Endocrinol.* **13**, 16 (1955).
93. Murphree, R. L., Warwick, E. J., Casida, L. E., and McShan, W. H., *J. Animal Sci.* **3**, 12 (1944).
94. Nalbandov, A. V., Moore, W. W., and Norton, H. W., *Endocrinology* **56**, 225 (1955).
95. Neher, C. M., and Zarrow, M. X., *Anat. Record* **106**, 556 (1950).
96. Neher, C. M., and Zarrow, M. X., *J. Endocrinol.* **11**, 323 (1954).
97. Odor, D. L., and Blandau, R. J., *Anat. Record* **97**, 400 (1947).
98. O'Mary, C. C., Pope, A. L., and Casida, L. E., *J. Animal Sci.* **9**, 499 (1950).
99. Pålsson, H., *Proc. 3rd Intern. Congr. Physiol. and Pathol. Animal Reproduction and Artificial Insemination Cambridge Sect. 1*, p. 112 (1956).
100. Papadopoulos, J. C., and Robinson, T. J., *Australian J. Agr. Research* **6**, 471 (1957).
101. Parker, C. H., *Phil. Trans. Roy. Soc. London* **B219**, 381 (1931).
102. Phillips, R. W., Fraps, R. M., and Frank, A. H., in "The Problem of Fertility" (E. T. Engle, ed.), p. 11. Princeton Univ. Press, Princeton, New Jersey, 1946.
103. Phillips, R. W., Simmons, V. L., and Schott, R. C., *Am. J. Vet. Research* **4**, 360 (1943).
104. Polovtzeva, V. V., and Fomenko, M. V., *Problemy Zhivotnovodstva* No. 5, 95 (1933).
105. Polovtzeva, V. V., and Fomenko, M. V., *Uspekhi Zooteh. Nauk* **3**, 51 (1936).
106. Quin, J. I., and van der Wath, J. C., *Onderstepoort J. Vet. Sci. Animal Ind.* **18**, 139 (1943).
107. Quinlan, J., and Maré, C., *Rept. Vet. Research S. Africa* **17**, 663 (1931).
106. Quinlan, J., Maré, C., and Roux, L. L., *Rept. Vet. Research S. Africa* **16**, 631 (1932).
109. Quinlan, J., Steyn, H. P., and de Vos, D., *Onderstepoort J. Vet. Sci. Animal Ind.* **16**, 243 (1941).
110. Radford, H. M., and Watson, R. H., *Australian J. Agr. Research* **6**, 431 (1955).
111. Riches, J. H., and Watson, R. H., *Australian J. Agr. Research* **5**, 141 (1954).
112. Richter, F., and Rittau, M., *Arch. Tierernähr. Tierzucht* **9**, 232 (1933); *Animal Breed. Abstr.* **1**, 106 (1933).
113. Robinson, T. J., *J. Agr. Sci.* **40**, 275 (1950).
114. Robinson, T. J., *J. Agr. Sci.* **41**, 6 (1951).
115. Robinson, T. J., *Biol. Revs. Cambridge Phil. Soc.* **26**, 121 (1951).
116. Robinson, T. J., *Nature* **170**, 373 (1952).
117. Robinson, T. J., *J. Endocrinol.* **10**, 117 (1954).
118. Robinson, T. J., *Endocrinology* **55**, 403 (1954).
119. Robinson, T. J., *Australian J. Agr. Research* **5**, 736 (1954).
120. Robinson, T. J., *J. Endocrinol.* **12**, 163 (1955).
121. Robinson, T. J., *J. Agr. Sci.* **46**, 37 (1955).
122. Robinson, T. J., *Australian J. Agr. Research* **7**, 194 (1956).
123. Robinson, T. J., *Australian J. Agr. Research* **9**, 693 (1958).
124. Robinson, T. J., unpublished data.
125. Robinson, T. J., and Moore, N. W., *J. Endocrinol.* **14**, 97 (1956).
126. Robinson, T. J., Moore, N. W., and Binet, F. E., *J. Endocrinol.* **14**, 1 (1956).
127. Robinson, T. J., Reardon, T. F., and Lamond, D., unpublished data, 1957.

128. Raux, L. L., *Onderstepoort J. Vet. Sci. Animal Ind.* 6, 465 (1936).
129. Santolucita, J. A., Clegg, M. T., and Ganang, W. F., *J. Animal Sci.* 16, 1096 (1957).
130. Schinkel, P. G., *Australian Vet. J.* 30, 189 (1954).
131. Schinkel, P. G., *Australian J. Agr. Research* 5, 465 (1954).
132. Sebott, R. G., and Phillips, R. W., *Anat. Record* 79, 531 (1941).
133. Starke, N. C., *Onderstepoort J. Vet. Sci. Animal Ind.* 22, 415 (1949).
134. Sykes, J. F., and Gole, G. L., *Mich. State Univ. Agr. Expt. Sto. Quart. Bull.* 26, 250 (1944).
135. Thibault, C., and Laplaud, M., *Camp. rend.* 224, 1786 (1947).
136. Thibault, C., Laplaud, M., and Ortavant, R., *Camp. rend. acad. agr. France* 34, 151 (1948).
137. Thibault, C., Ortavant, R., and Laplaud, M., *Ann. endocrinal. (Paris)* 9, 83 (1948).
138. Underwood, E. J., and Shier, F. L., *J. Dept. Agr. W. Australia* 19, 176 (1942).
139. Underwood, E. J., Shier, F. L., and Davenport, N., *J. Dept. Agr. W. Australia* 21, 1 (1944).
140. Wallace, L. R., *J. Agr. Sci.* 45, 60 (1954).
141. Wallace, L. R., *Proc. Ruakoro Farmers' Conf. Week* p. 38 (1955).
142. Wallace, L. R., Lambourne, L. J., and Sinclair, D. P., *New Zealand J. Sci. Technol.* A35, 421 (1954).
143. Warbritton, V., *J. Morphol.* 56, 181 (1934).
144. Warbritton, V., and McKenzie, F. F., *Missouri Univ. Agr. Expt. Sto. Research Bull. No.* 257 (1937).
145. Warwick, E. J., *Proc. Soc. Exptl. Biol. Med.* 63, 560 (1946).
146. Watson, R. H., *Australian Vet. J.* 28, 1 (1952).
147. Watson, R. H., *Australian J. Agr. Research* 4, 349 (1953).
148. Watson, R. H., and Radford, H. M., *Australian Vet. J.* 31, 31 (1955).
149. Williams, S. M., Garrigus, U. S., Norton, H. W., and Nalbandov, A. V., *J. Animal Sci.* 15, 984 (1956).
150. Wiltbank, J. N., and Casida, L. E., *J. Animal Sci.* 15, 134 (1956).
151. Wintenberger, S., *Ann. Zootech. Paris* 2, 269 (1953).
152. Wintenberger, S., *Ann. endocrinal. (Paris)* 16, 383 (1955).
153. Wintenberger-Torres, S., *Proc. 3rd Intern. Congr. Physiol. and Pathol. Animal Reproduction and Artificial Insemination Cambridge Sect. 1*, p. 62 (1956).
154. Yeates, N. T. M., *Nature* 160, 429 (1947).
155. Yeates, N. T. M., *J. Agr. Sci.* 39, 1 (1949).
156. Yeates, N. T. M., in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 1, p. 363. Butterworths, London, 1954.
157. Zavadovskii, M. M., "Hormonal Stimulation of Multiple Fecundation in Sheep." Moscow, 1941. (English summary.)
158. Zavadovskii, M. M., "Reserves of Animal Breeding. Stimulation of Multiple Fecundation in Livestock." Moscow, 1945. (English summary.)
159. Zavadovskii, M. M., and Paduceva, A. L., *Trudy Dinam. Razvit.* 11, 64 (1939).
160. Zavadovskii, M. M., and Paduceva, A. L., *Doklady Acad. Sel'skhoz. Nauk.* No. 4, 35 (1939).
161. Zavadovskii, M. M., and Paduceva, A. L., *Socialist Zivotn. No.* 6, 48 (1939).

CHAPTER 10

The Estrous Cycle of the Sow

J. M. BODA

	<i>Page</i>
I. Introduction	335
II. Prepuberal Development	336
III. The Attainment of Puberty	337
IV. The Estrous Cycle	338
A. The Length of the Cycle	338
B. Estrus	339
1. Duration of Estrus	339
2. Time of Estrus in Relation to Parturition	340
3. Time of Estrus in Relation to Weaning	340
4. External Manifestations of Estrus	341
C. The Cyclic Changes during the Estrous Cycle	341
1. Ovary	341
2. Uterus and Oviduct	345
3. Vagina	346
4. Anterior Pituitary	347
5. Other Changes	349
D. Ovulation	349
1. Time of Ovulation in Relation to Estrus	349
2. Rate of Ovulation	350
E. Experimental Modification of the Estrous Cycle	352
1. Use of Gonadotropins	352
2. Use of Gonadal Hormones	353
References	355

I. INTRODUCTION

The classic investigations by the laboratories of Corner and McKenzie in the 1920's and early 1930's provide an insight into the reproductive cycle of the sow. However, except for the inheritance of reproductive ability furnished by the Regional Swine Breeding Project and the excellent nutritional and physiological studies by Casida and co-workers at the University of Wisconsin, later work has been rather sporadic. In general, the more recent techniques have not been applied in the sow to a marked extent. This is somewhat surprising, since the sow is unique among the larger domestic animals in being polytocous, and since maximum reproductive performance in this species is of considerable economic importance. The discussion to follow, then, is an attempt to bring together, in an organized fashion, the available information regarding the estrous cycle of this particular species.

II. PREPUBERAL DEVELOPMENT

As an introduction to the physiological alterations associated with the estrous cycle that are not initiated until the attainment of sexual maturity, it is desirable to review several investigations which have provided an insight into the prepuberal development of the reproductive apparatus.

Casida (12) studied the ovaries of gilts ranging in age from 1 to 112 days. Egg cords were present in the outer peripheral zone of the ovary until the 4th week of age. Thereafter, until the 7th week, when multilayered follicles began to appear, primary follicles predominated in both the outer and inner zones. The number of layers of granulosa cells increased and, when 14 to 20 were present, antrum formation occurred. Vesicular follicles were observed in pigs 11 weeks old, but were not found consistently or in large numbers until the 15th week. An increase in the vascularity of the medullary region of the ovary occurred at the time of multilayered follicle formation, and an increase in the blood supply to the theca interna of individual follicles was associated with antrum formation. Various gonadotropic preparations were ineffective before the formation of vesicular follicles, but thereafter gonadotropin administration induced follicular development, ovulation, and the development of corpora lutea. In a similar study, Wetli (64) investigated the development of prepuberal gilt ovaries and distinguished three periods of differentiation: a period of primary follicle formation during the first 2 weeks after birth, one of secondary follicular development lasting from the 3rd to 7th week, and a third period of tertiary follicle development lasting from the 7th week until puberty. In the first period, the ovary was embryonic in character. The rete was still large and the deeper layers of the cortex contained only primary follicles isolated from the ovarian vesicles in the cortical surface. Frequent oögonia formation was observed in the upper cortical layers. During the second period, secondary follicles appeared to form in bursts. The tunica albuginea developed along with a general proliferation of the connective tissue framework. The third period was characterized by a rapid increase of ovarian size and by a cyclic development and involution of the follicles. The decisive differentiation appeared to take place between the 8th and 11th weeks of age. Thereafter, further formation of oögonia was not observed.

The changes in the gonadotropic content of the pituitary from birth to maturity have received some attention (30). These investigators assayed the total gonadotropic hormone content of the anterior pituitaries obtained from gilts and sows ranging in age from birth to 1330 days,

using the growth response of the testis of immature cockerels receiving standard doses of the dehydrated glands. They observed a highly significant positive correlation between the dry weight of the gland and the age of the animal. The total hormone content of the gland also increased with age; however, the gonadotropic content per unit of body weight was very high at birth, declined until puberty, and remained relatively constant thereafter. It was suggested that the apparent drop from birth to sexual maturity was more likely due to a relative shift in the proportion of FSH and LH rather than to an actual reduction in the secretory rate of total gonadotropin. On this basis, it was postulated that the onset of puberty was due not to a gradual increase in the total gonadotropic hormone secretion to a critical level, but rather to a shift in the proportion of FSH to LH. In other words, at birth, pituitary secretion is predominantly FSH, but, with increasing age, FSH is markedly reduced and LH increases somewhat. Puberty occurs, these workers concluded, when the proper proportion of FSH and LH is attained.

III. THE ATTAINMENT OF PUBERTY

The gilt usually attains sexual maturity at about 7 months of age. However, as discussed below, there is considerable variation, determined, in part, by genetic and nutritional variability and probably by the season of the year at which gilts are farrowed.

Breed differences in the age at sexual maturity are not marked. Burger (11) found no difference between the Large Black and Large White breeds in this respect. There was, however, a highly significant difference between families. Self *et al.* (49) observed no difference between Chester White and Poland China gilts. Phillips and Zeller (43) reported the average ages of first estrus for small- and large-type Poland China gilts as 208 and 199 days, respectively. This difference is not statistically significant, unless adjustments to a common body weight are made, in which case, age at puberty for the small type was delayed.

Inbreeding delays sexual maturity by several weeks. Warnick *et al.* (62) found that the average age at puberty of inbred lines of the Chester White and Yorkshire breeds and an inbred line originating from a Chester White Yorkshire cross (coefficients of inbreeding, 0.27 to 0.29) was about 8 months, in comparison with 7 months for outcrossed gilts. Squiers *et al.* (55) and Foote *et al.* (27) reported similar findings in inbred lines in comparison with line-crossed gilts, and Squiers *et al.* (55) estimated that for each 10% increase of inbreeding of parent lines, sexual maturity was delayed about 13 days.

Most of the delay of sexual maturity associated with inbreeding has

been attributed to the marked variability in thriftiness and general health (62). Burger (11) found, as for other species, that retarding the growth rate by limiting food intake will delay sexual maturity. In a controlled feeding experiment, puberty was delayed an average of 46 days in a group of gilts fed so as to gain at half the rate of the full-fed controls. Presumably, the ration used was adequate in vitamins A, C, and D, but was deficient in protein and total energy. It has been reported that vitamin B₁₂ deficiency will delay puberty (32). Self *et al.* (49) studied the effects of limited and self-feeding on puberty and concluded that the "fatness" of the self-fed gilts delayed the age of puberty by about 2 weeks, in comparison with that of gilts fed at only two-thirds the self-fed rate. However, these results are not directly comparable with those of Burger (11) in that limited feeding was much less severe and growth rate was reduced a relatively small amount.

In addition to these genetic and nutritional effects, the time of farrowing, in relation to the season of the year, may determine the age at first estrus. Robertson *et al.* (45) reported that gilts born late in the spring farrowing season tend to reach puberty sooner than those born earlier in the year. Wiggins *et al.* (66) reached a similar conclusion after examining the reproductive tracts and ovaries of nearly 3000 market gilts of unknown age and 113 gilts of known age at the time of slaughter. In the former group, the average incidence of prepuberal sexual development was lowest in April and highest in October. In the gilts of known age, the percentage of immature gilts in the group born early in the spring was significantly higher than in those born later, even though the latter were younger at the time of slaughter. Schmidt and Bretschneider (47) also report a seasonal effect on the age at puberty. On the other hand, Self *et al.* (49), found no correlation between puberal age and time of birth. However, the farrowing dates were all within 32 days, thus limiting the possibility of an observable seasonal effect.

IV. THE ESTROUS CYCLE

A. The Length of the Cycle

The average length of the estrous cycle of gilts and sows is about 21 days. Table I presents the estrous cycle length of the sow as reported by various investigators.

Burger (11) found no correlation between the duration of estrus and the length of the cycle. There was a statistically significant difference in cycle length between the Large Black and Large White breeds. Robertson *et al.* (45), however, found no difference between the Chester White and Poland China breeds, although in a later paper (49) this group of investigators reported that the variation around the mean was

significantly greater for Chester White (SD 1.6) than for Poland China gilts (SD about 1.0)

TABLE I
THE ESTROUS CYCLE LENGTH IN THE SOW

Authority	Estrous Cycle Length	
	Mean and SD	Range
Struve (57)	20.66 \pm 2.36	15 to 30 days, 75% between 18 and 23 days
McKenzie and Miller (42)	21 to 22	
Krallinger (38)	21 \pm 2½	
Robertson <i>et al</i> (45)	20.5 \pm 1.48 (Chester Whites)	
	21.0 \pm 0.95 (Poland China)	
Burger (11)	21.7 \pm 2.32 (Large Blacks)	
	20.9 \pm 3.52 (Large Whites)	
Schmidt and Bretschneider (47)	20.5	11 to 41 days, 77% between 17 and 25 days

Although "silent heat" periods are relatively rare in swine, they occur occasionally. Burger (11) estimated the incidence of "silent heat" as about 1.5% in over 950 cycles studied. The occurrence of an occasional silent heat period may explain the great range of estrous cycle lengths reported by Schmidt and Bretschneider (47). In other words, a 41-day cycle length may represent two consecutive estrous cycles separated by a "silent heat" period overlooked by these investigators.

McKenzie and Miller (42) reported little difference in cycle length with increasing sexual age.

B Estrus

1 Duration of Estrus

Even though the onset and disappearance of estrus, as measured by vulvar swelling, interest in the boar, etc., is gradual (11), the actual period of estrus ("heat"), when the sow will accept the boar, has been quite accurately determined. McKenzie and Miller (42) reported the average duration of estrus as 40 to 46 hours in the normal cycling sow. There was little difference between the length of the first estrus at puberty and that of subsequent estrous periods, except for the first period after weaning, which was longer, averaging 65 hours. Burger (11) also reported that the duration of estrus did not vary with advancing sexual age, and that the first four periods after puberty did not differ significantly. There was a difference between breeds (and families), and the first heat periods following parturition and weaning were longer than those preceding conception. The mean durations, in hours, for the

Large Black and Large White breeds were as follows: first estrus at puberty, 58.50 and 48.65; sow estrus, 68.19 and 49.91; postpartum estrus, 68.20 and 65.42; and first postweaning estrus, 65.05 and 57.84, respectively. The individual lengths of the estrous periods varied from 15 to 96 hours. In general, the estrous periods of the Large Black breed were significantly longer. The onset of estrus occurred just as often during the "day" (from 6 A.M. to 3 P.M.) as during the "night" (from 6 P.M. to 3 A.M.). Coitus, with either intact or vasectomized boars, did not influence the duration of estrus. Schmidt and Bretschneider (47) reported that in 32 sows, observed over a period of 2½ years, the duration of estrus averaged 2.47 ± 1.30 days with a range of 0.5 to 10.0 days. They observed no breed differences; estrous periods were shortened, however, when the average monthly temperature exceeded 16°C.

2. Time of Estrus in Relation to Parturition

Usually, the sow exhibits a nonfertile estrous period approximately 2 days after parturition. However, there seems to be considerable individual variation in this respect. Baker *et al.* (6) reported that only 17 of the 29 sows studied came into heat within 1 to 3 days following parturition, and that none of the sows in heat conceived when bred. Burger (11), on the other hand, found that only 3 of 88 sows failed to exhibit a postpartum estrus. The intervals between parturition and the onset of estrus were 41.3 ± 9.44 hours for the Large Black breed and 47.59 ± 16.42 hours for the Large White breed, a difference which was not statistically significant. The interval between parturition and estrus was independent of the number of pigs farrowed. None of the 13 sows bred at the postpartum estrus conceived. Warnick *et al.* (61) observed postpartum heat in only 18 of the 36 sows investigated. Two of the sows exhibiting estrus ovulated, and fertile ova were recovered from the reproductive tracts at slaughter. Sows which did not exhibit heat did not ovulate. The average interval from parturition to the onset of estrus tended to be longer, although the difference was not statistically significant, and the average duration of the postpartum estrus was significantly longer in nonsuckled (60 hours) than in suckled sows (20 hours). Since ovarian development was not marked (the maximum follicular diameter was 5 to 7 mm.) and the corpora lutea of pregnancy were undergoing regression, these investigators suggest that an extra-ovarian source of estrogen is responsible for the postpartum estrus.

3. Time of Estrus in Relation to Weaning

Estrus is normally inhibited during lactation, except for the usual postpartum period, although an occasional heat period may occur in

some sows before weaning (2, 11, 28, 38). In such cases, ovulation occurs, fertile ova are shed, and conception will occur if the sows are bred (11).

The interval between the end of lactation and the onset of heat is quite variable. Krallinger (38) found an average interval of 7 days in 147 observations, while Allen *et al.* (2) observed an average interval of 9.6 days. Burger (11) reported the intervals for the Large Black and Large White breeds as 16.1 ± 21.43 and 7.85 ± 11.07 , respectively. The variability was so great, however, that the mean values have little significance. No relationship has been found between the 4-week weight of the litter or the decline of the sow's weight and the return of the postweaning estrous cycle (38). Thus, the demands of lactation do not influence this interval. This finding was confirmed by Burger (11), who found no correlation between the return of estrus and the length of the nursing period, the litter size at weaning, or the loss of the sow's weight during lactation.

The removal of the suckling stimulus for a sufficiently long period re-initiates the cycle. Baker *et al.* (6) removed the litter from sows either at parturition or within two days after farrowing. About 76% came into heat within 8 to 16 days after farrowing. The estrous period lasted an average of 2.82 days, and 92.5% of the 197 ova recovered after slaughter were fertile. Burger (11) was unable to induce estrus in lactating sows by removing the litter overnight.

4. External Manifestations of Estrus

The most evident external manifestations of estrus in the sow are characteristic sexual behavior patterns and an increase in the size of the vulva. The sexual behavior of the sow is described in detail by Burger (11). Apparently, the most consistent behavior indicative of estrus is submission to "riding" by other animals, either male or female. The fluctuations of the size of the vulva with the estrous cycle have been studied quantitatively (11, 42). Burger (11) observed that the vulva began to enlarge 3 to 4 days before estrus and that the onset of estrus could be accurately predicted to occur on the 4th day after the first positive indications of vulvar swelling. In prepuberal gilts, however, vulvar enlargement often occurs several weeks before the puberal estrus.

C. The Cyclic Changes during the Estrous Cycle

1. Ovary

The classic paper by Corner (17) described in detail the morphological changes of the ovary throughout the estrous cycle. More re-

cently, Burger (11) reinvestigated the gross aspects of the development and regression of the ovary during the cycle, describing these changes as follows.

During diestrus, the ovarian weight increases from the 3rd until the 12th day after the onset of estrus. This is due, primarily, to the growth of the developing corpora lutea, which enlarge shortly after ovulation and reach a maximum of about 11 mm. on the 15th day of the cycle. They then decrease as regression proceeds. The corpora gradually change in color from a dark red on the 3rd day to a pale purple by the 15th day of the cycle. Between the 15th and 18th days they are yellowish-cream, and with advancing age they become white. At the 3rd day, the central cavities of the corpora lutea are filled with dark-red blood clots, which may be replaced by connective tissue plugs by the 6th day, or by a yellowish fluid which may persist up until the 15th or 18th day of the cycle. A marked vascularity of the corpora is plainly evident from the 6th to the 18th day of diestrus. The corpora lutea of the preceding cycle do not change in diameter appreciably during diestrus, but they rapidly undergo involution at the onset of estrus and virtually disappear when about 40 days old. The average diameter of the large follicles increases until the 18th day, reaching a maximum of approximately 9 mm. The total number of grossly visible follicles does not change during diestrus. This statement is in variance with the findings of Robinson and Nalbandov (46) who have reported a sharp rise in follicular numbers at the 8th day of the cycle.

At estrus, the corpora lutea undergo a rapid regression, the average diameter being reduced nearly 50%. The most rapid rate of involution coincides with ovulation. The definitive follicles enlarge until the 18th hour after the onset of estrus, but thereafter size changes are variable. The color of the mature follicle has been described as "sea-shell pink." This may be due to the fine network of blood vessels overlying the follicular surface. A transparent area at the apex of the follicle is an indication of immediate rupture. Size alone is no criterion, since, at ovulation, the follicles may vary several millimeters in diameter. Congestion of the arterial system and extravasation of blood into the follicles, giving rise to hemorrhagic follicles, occur frequently in the sow. At ovulation, the follicle flushes and rupture may be detected by the collapse of the follicle walls and the appearance of a reddish fluid over the surface. The rupture point can be distinguished until the 12th day after the onset of estrus. The immature follicles at estrus are very small, a reduction in mean diameter having occurred during proestrus.

The histological changes of the follicle and corpus luteum of the

sow during the estrous cycle have been described by Corner (15, 17). In brief, 2 or 3 days before the onset of estrus, the definitive follicle begins to enlarge rapidly. At this time, hypertrophy of the theca interna and partial dissolution of the cumulus oöphorus occur, to the extent that the ovum is nearly freed of follicular anchorage. The ovum itself undergoes the first stages of maturation. The nucleus moves toward the periphery of the cell, where it undergoes mitosis, and the first polar body is expelled. At ovulation, the first polar body has been discharged and the second polar spindle has formed. Spalding *et al.* (52) studied the development of the ovum immediately preceding, during, and after ovulation.

With the collapse of the follicle at ovulation, the granulosa remains intact, except for the loss of the cumulus oöphorus with the ovum. The granulosa cells undergo hypertrophy and become filled with lipid material developing into the "lutein" cells of the mature corpus luteum. Blood vessels from the theca interna ramify throughout the developing corpus, carrying with them the lipid-containing cells of the theca interna, which become lodged throughout the corpus between the lutein cells of granulosa origin. By the 7th day after the onset of estrus, the corpus luteum is fully differentiated. The lutein cells of the mature corpus are very conspicuous, being 30 to 40 μ in diameter. The cells originating from the theca interna are smaller (10–25 μ) and are packed with small vacuoles and fat droplets. The lutein cells are held by a framework of reticular connective tissue which arises from the capillary endothelium (16). Regression of the corpora lutea begins on about the 15th day of the estrous cycle. The lutein cells undergo a rapid degeneration, become vacuolated with pyknotic nuclei. The blood capillaries collapse, and, as regression proceeds, the connective tissue becomes thicker and more dense. The cells of thecal origin do not degenerate as abruptly but may persist for several weeks, enmeshed within the connective tissue. When about 6 weeks old, the corpus luteum becomes indistinguishable from old atretic follicles.

Chemical (9) and cytochemical (7) studies of the ovary have revealed distinct changes in the composition of the follicle, and particularly of the corpus luteum, during the estrous cycle. Bloor *et al.* (9) reported that the phospholipid content of the corpus luteum follows closely the gross and histological changes of development and regression. Thus, in the first days after ovulation, the relative increase is small, but from the 5th through the 10th day of the cycle the phospholipid content rises rapidly. With regression, the content falls rapidly after the 14th day. Free cholesterol increases progressively to the 18th day and then

remains at a fairly constant level. Bound cholesterol remains low until the 14th day and then rises rapidly with retrogression of the gland. The percentage increase of total cholesterol is slow until the regression of the corpus, but thereafter the increase is rapid, probably because this material is not as readily reabsorbed as are other constituents of the gland. These results indicate that the phospholipid content varies with the physiological activity of the gland during development and regression. Boyd and Elden (10) report similar findings. Barker (7) employed a number of cytochemical tests (sudanophilia, reactivity to carbonyl reagents, birefringence, fluorescence, and acetone solubility), believed to characterize steroid-secreting tissues, in an investigation of the sow's ovary during the estrous cycle. Such "lipids" were present only in the ova of the primordial follicles. When the follicles became vesicular, the basal cells of the granulosa and the cells of the theca interna acquired lipids. These investigators conclude that such cells represent the sites of ovarian hormone production, although with the methods employed the individual steroids could not be characterized. The "lipid" cells were largest, but individual droplets smallest, in the thecal cells just prior to ovulation, indicating an active secretory phase. During the first 3 days after ovulation, all of the lutein cells contained large lipid droplets; however, they were most numerous in those derived from the theca interna and in the granulosa cells adjacent to the central coagulum. From the 4th to the 14th day of the cycle, the over-all concentration of lipid was lower than during metestrus. The droplets remained numerous in the theca lutein cells. During late diestrus and proestrus, from the 15th day of the cycle until estrus, the lipid droplets in the regressing corpus luteum, as in the theca interna of atretic and cystic follicles, and in the interstitial tissue of the ovary became insoluble in acetone, although they continued to respond positively to the other tests employed. It was suggested that these may be steroid condensation products.

Several investigators have determined the hormone content of the sow's ovary in relation to the stage of the estrous cycle. Elden (24) reported that the progestin and estrogen content of the corpus luteum was high shortly after ovulation and low during regression. In a more definitive study, Boyd and Elden (10) found that the estrogen content of the gland increased from slightly less than 3 R.U. (rat units) per 100 g. of fresh tissue after ovulation to a maximum of about 6 R.U. at the 15th to 18th day of the cycle. It then declined as the corpus luteum degenerated. The progestin content exhibited two peaks, one shortly after ovulation (4 rabbit units), and the second 12 to 15 days after estrus

(over 5 rabbit units). Kimura and Cornwell (35) found appreciable quantities of progesterin in the corpus luteum before the gland was fully differentiated. The content increased to a maximum on the 15th day of the cycle and then declined. Hisaw and Zarrow (29) reported the presence of small amounts of relaxin in the ovary of the sow during the luteal phase of the cycle but none during the follicular phase. Relaxin was absent in the prepuberal gilt ovary.

2. Uterus and Oviduct

The histological changes of the uterus, as described by Corner (17) and confirmed by McKenzie (41), can be summarized as follows. During estrus, the surface epithelium is pseudostratified and varies in height from 25 to 30 μ . Mitotic figures occur frequently in the surface epithelium but are not numerous in the uterine gland cells. The stroma is edematous and the subepithelial connective tissue is invaded by many neutrophilic, polymorphonuclear leucocytes. During the first week after ovulation, the epithelial cells undergo hypertrophy. The pseudostratified condition is replaced by a high columnar type of epithelium which attains a height of 35 to 50 μ toward the end of this period. Three to four days after ovulation, the more superficial gland cells begin to multiply. By the end of the week, numerous mitotic figures are evident in the basal gland cells. Eosinophilic, polymorphonuclear leucocytes, which are always present to some extent in the stroma, increase greatly in number but do not invade the epithelium. Stromal edema is reduced. In mid-diestrus, 8 to 10 days after ovulation, the high columnar type of epithelium persists, the nuclei occupying a central position in the cell. The surface cells are so arranged as to give a wavy or hilly appearance, a condition characteristic of this stage of the cycle. Here and there, abnormal, degenerating cells with pycnotic nuclei are compressed between the normal, actively secreting cells. At this period, the invasion of the more superficial portion of the stroma by eosinophilic leucocytes is at its height. During late diestrus, 10th to 15th day of the cycle, the surface epithelium reverts to a low columnar type, 15 to 20 μ high. Cytoplasmic processes, 3 to 8 μ in height, are extruded from each cell, which, according to Corner (17), are not cilia. [Snyder and Corner (51) and McKenzie (41) have reported that cilia are not present on the surface epithelium at any stage of the cycle, but that they are always present in the uterine glands. Their number and activity do not appear to fluctuate with the estrous cycle, and the former investigators suggested that transportation of the ova was not their primary function.] The excess numbers of eosinophilic leucocytes disappear from the

stroma. During late diestrus and proestrus, the surface epithelial cells are low columnar or nearly cuboidal. The cytoplasmic processes have disappeared. Marked vacuolar degeneration of the epithelial cells is characteristic of this stage of the cycle. A large number of neutrophilic leucocytes accumulates in the subepithelial stroma, and edema is initiated.

A number of investigators have studied the changes of uterine motility during the estrous cycle. Seekinger (48) and Keye (33) observed a cyclic variation of amplitude and rate of contraction of isolated strips of the sow's uterus. From these findings, Corner (18) presented the theory, now fairly well established, that the uterine and tubal contraction cycles represent a peristaltic mechanism serving to transport the ova. In general, the amplitude of contraction is greatest during estrus, but the major contraction waves occur less frequently. These findings have been confirmed and extended by other investigators: King (36) found that the work capacity of the isolated, loaded muscle was greatest during estrus and least at the height of the luteal phase of the cycle; Whitelaw (65) observed a cyclic variation in the contraction of the oviduct; and Csapo and Corner (20) reported that spontaneous uterine motility at estrus is characterized by relatively infrequent contractions of large amplitude followed by quick, complete relaxation, while during the luteal phase of the cycle, the isolated uterus develops spontaneous "contracture." Adams (1) presented some data suggesting that the uterine muscle of the sow responds to pituitrin with contraction and to adrenaline with relaxation more intensely during the luteal phase of the estrous cycle. Pomeroy (44) recently showed that the rate of passage of the ovum through the oviduct is relatively rapid in the sow, the ova entering the uterus between 24 and 48 hours after ovulation. He suggested that this swift passage is due to the high progesterone titers of the sow resulting from the numerous corpora lutea.

3. Vagina

McKenzie (41) and Wilson (67) described the histological changes of the vagina during the estrous cycle. The vaginal stroma contains a small number of leucocytes throughout the cycle. However, cyclic variations do occur. Leucocyte numbers increase during the first 4 days after estrus and then decrease by mid-diestrus. The vaginal epithelium is the stratified squamous type. It varies in height with the cycle, increasing to a maximum at estrus and then progressively decreasing to a low between the 12th and 16th days of the cycle. A heavy sloughing of the superficial layers begins at about the 4th day and is completed

by the 16th day after estrus. Leucocytes invade the epithelium during late diestrus and increase in numbers, reaching a maximum during metestrus when they are removed by desquamation of the epithelium.

During estrus the pH of the vaginal mucus may be lower and vaginal temperatures higher (47).

4. Anterior Pituitary

The morphological changes of the anterior pituitary of the sow, in relation to the estrous cycle, have received little attention since the publication of a paper by Cleveland and Wolfe in 1933 (13). These investigators collected the pituitaries of 41 sows killed at selected periods after the onset of estrus. The exact stage of the cycle was estimated by examining the gross and histological appearance of the ovaries and reproductive tracts. They described definite changes in the histology of the anterior pituitary associated with various stages of the cycle. During proestrus, from the 15th to 20th days, eosinophilic cells predominate, and the number of cytoplasmic granules appears to increase progressively during this period. A few nearly agranular eosinophils, typical of the luteal phase of the cycle, are observed. Basophils are conspicuous and the majority are filled completely with granules. There may be a reduction of the numbers of chromophobes during late proestrus. During estrus, eosinophils are numerous and packed with granules. The number of granules per basophil is markedly reduced, and the number of nongranular basophils increases. The relative number of chromophobes is also reduced markedly. The early luteal phase of the cycle, from 0 to 7 days after estrus, is characterized by a general reduction of eosinophilic, cytoplasmic granules in the alpha cells, and an increase of basophilic granules in the beta cells. During the most active luteal phase, 8th to 11th days of the cycle, there is little reduction in the number of eosinophils, but the majority contain few granules. The number of basophils is diminished, and many contain granules which tend to accumulate around the nucleus. Many cells of this latter type undergo regression and may be transformed into chromophobes. The late luteal phase, 10th to 15th day of the cycle, is characterized by regressive changes of both the eosinophils and basophils and a relative increase in the numbers of chromophobes.

These investigators attempted to correlate their histological observations with earlier studies regarding the changes in the relative capacity of the gland tissue to induce ovulation when injected into rabbits (68). In general, they could not observe a clear-cut association between the hormonal activity of the gland and the relative number and cytological appearance of either the basophils or eosinophils.

The cyclic fluctuations of the total gonadotropic hormone content of the anterior pituitary have received more attention than the morphological changes associated with the reproductive cycle. As previously mentioned, Wolfe (68) determined the relative ability of fresh anterior pituitary tissue, collected from sows at various stages of the estrous cycle, to induce ovulation in the rabbit. He found that the equivalent of 1 mg. of fresh tissue, obtained during the follicular phase of the estrous cycle, would induce ovulation consistently, while 10 and 40 mg. were required when the tissues were collected during estrus or during the luteal phase of the cycle, respectively. One interpretation of these results was the suggestion that since ovulation was induced with most difficulty with pituitary tissue collected during the luteal phase of the cycle, luteinizing hormone was not involved with follicular rupture. Faiermark and Singerman (26) estimated the total gonadotropic potency of sow pituitaries by implanting the fresh tissue into mice and reported similar results, namely, that the gonadotropic content was highest during proestrus, fell sharply at estrus, and was very low during diestrus. Robinson and Nalbandov (46) determined the total gonadotropic hormone content of the anterior pituitaries of 33 normally cycling sows killed at definite intervals after the onset of estrus. The glands were assayed by measuring the increases of testicular weights of chicks receiving standard doses of the dehydrated ground tissue. An estimate of the total surface area of the ovaries of the donor sows occupied by follicles, a so-called "follicular index," was used to evaluate ovarian follicular development. This was done in an attempt to interpret the functional significance of fluctuations of the total gonadotropic hormone content of the pituitary in terms of the resultant activity of the target organ. From the 1st through the 7th days of the estrous cycle, the gonadotropic content of the pituitary and the follicular index were low. The authors suggested that the low gonadotropic level during estrus may result from pituitary inhibition by estrogen and a shift in the relative proportion of FSH and LH. On the 8th day of the cycle, the gonadotropic content increased sharply and remained high until the 20th day. The follicular index showed the same trend, primarily because of an increase in numbers of follicles until the 14th day, and thereafter because of an increase in follicular size. From the 20th day until estrus, there was a pronounced drop of gonadotropic content and follicular numbers. In general, there was a statistically significant positive correlation between the pituitary gonadotropin content, the ovarian follicular index, and the day of the estrous cycle. This finding is interpreted as suggesting that the total gonadotropic content of the gland is indicative

of the actual secretory rate of this hormone. It is difficult to interpret the significance of fluctuations in total gonadotropic content of the pituitary in terms of the hormonal regulation of the estrous cycle. A determination of the individual changes in FSH and LH at various phases of the cycle should help to clarify this aspect of reproductive physiology in the sow.

5 Other Changes

McKenzie (41) described the histological changes of the vestibular epithelium of the vulva in some detail. In general, the height of the epithelium reaches a maximum during estrus, and desquamation and leucocytic infiltration are most marked on the 2nd to 4th days after heat. Altmann (3) studied the cyclic variations of the vaginal smear, activity as recorded with a pedometer, body temperature, heart rate, and salivation through a parotid fistula. Activity increased during proestrus, reached a maximum at the end of estrus, and then declined to a low level immediately following estrus. In the other measurements there were no apparent differences which could be correlated with a definite stage of the cycle, except for a possible reduction of salivation during estrus. Curtis (21) also recorded the activity of sows with a pedometer and reported that spontaneous activity, particularly at night, was greatly increased during estrus.

D Ovulation

1 Time of Ovulation in Relation to Estrus

Ovulation in the sow occurs during the latter part of estrus (11, 19, 40) and is spontaneous, being independent of coitus (19). Specific changes in the histological appearance of the vaginal smear, the pH of the vaginal mucus, or of the rectal or vaginal temperature during estrus cannot be used to establish the time of ovulation (47). Burger (11) investigated, in some detail, the time of ovulation in relation to the onset of estrus by examining the ovaries of gilts sacrificed at 6 hour intervals during estrus; he reported that ovulation occurs well into the second half of the estrous period. There was a highly significant breed difference in the time of ovulation, which was positively correlated with the duration of estrus. Thus, the mean durations of estrus and the interval between the onset of estrus and ovulation for the Large Black and Large White breeds were 62.5 and 42-54 hours, 47.6 and 18-36 hours, respectively. It was calculated that ovulation was delayed 6 hours for each 7.8 hour extension of estrus.

Burger attempted to determine the length of time required for all

The cyclic fluctuations of the total gonadotropic hormone content of the anterior pituitary have received more attention than the morphological changes associated with the reproductive cycle. As previously mentioned, Wolfe (68) determined the relative ability of fresh anterior pituitary tissue, collected from sows at various stages of the estrous cycle, to induce ovulation in the rabbit. He found that the equivalent of 1 mg. of fresh tissue, obtained during the follicular phase of the estrous cycle, would induce ovulation consistently, while 10 and 40 mg. were required when the tissues were collected during estrus or during the luteal phase of the cycle, respectively. One interpretation of these results was the suggestion that since ovulation was induced with most difficulty with pituitary tissue collected during the luteal phase of the cycle, luteinizing hormone was not involved with follicular rupture. Faiermark and Singerman (26) estimated the total gonadotropic potency of sow pituitaries by implanting the fresh tissue into mice and reported similar results, namely, that the gonadotropic content was highest during proestrus, fell sharply at estrus, and was very low during diestrus. Robinson and Nalbandov (46) determined the total gonadotropic hormone content of the anterior pituitaries of 33 normally cycling sows killed at definite intervals after the onset of estrus. The glands were assayed by measuring the increases of testicular weights of chicks receiving standard doses of the dehydrated ground tissue. An estimate of the total surface area of the ovaries of the donor sows occupied by follicles, a so-called "follicular index," was used to evaluate ovarian follicular development. This was done in an attempt to interpret the functional significance of fluctuations of the total gonadotropic hormone content of the pituitary in terms of the resultant activity of the target organ. From the 1st through the 7th days of the estrous cycle, the gonadotropic content of the pituitary and the follicular index were low. The authors suggested that the low gonadotropic level during estrus may result from pituitary inhibition by estrogen and a shift in the relative proportion of FSH and LH. On the 8th day of the cycle, the gonadotropic content increased sharply and remained high until the 20th day. The follicular index showed the same trend, primarily because of an increase in numbers of follicles until the 14th day, and thereafter because of an increase in follicular size. From the 20th day until estrus, there was a pronounced drop of gonadotropic content and follicular numbers. In general, there was a statistically significant positive correlation between the pituitary gonadotropin content, the ovarian follicular index, and the day of the estrous cycle. This finding is interpreted as suggesting that the total gonadotropic content of the gland is indicative

of the actual secretory rate of this hormone. It is difficult to interpret the significance of fluctuations in total gonadotropic content of the pituitary in terms of the hormonal regulation of the estrous cycle. A determination of the individual changes in FSH and LH at various phases of the cycle should help to clarify this aspect of reproductive physiology in the sow.

5. *Other Changes*

McKenzie (41) described the histological changes of the vestibular epithelium of the vulva in some detail. In general, the height of the epithelium reaches a maximum during estrus, and desquamation and leucocytic infiltration are most marked on the 2nd to 4th days after heat. Altmann (3) studied the cyclic variations of the vaginal smear, activity as recorded with a pedometer, body temperature, heart rate, and salivation through a parotid fistula. Activity increased during proestrus, reached a maximum at the end of estrus, and then declined to a low level immediately following estrus. In the other measurements there were no apparent differences which could be correlated with a definite stage of the cycle, except for a possible reduction of salivation during estrus. Curtis (21) also recorded the activity of sows with a pedometer and reported that spontaneous activity, particularly at night, was greatly increased during estrus.

D. *Ovulation*

1. *Time of Ovulation in Relation to Estrus*

Ovulation in the sow occurs during the latter part of estrus (11, 19, 40) and is spontaneous, being independent of coitus (19). Specific changes in the histological appearance of the vaginal smear, the pH of the vaginal mucus, or of the rectal or vaginal temperature during estrus cannot be used to establish the time of ovulation (47). Burger (11) investigated, in some detail, the time of ovulation in relation to the onset of estrus by examining the ovaries of gilts sacrificed at 6-hour intervals during estrus; he reported that ovulation occurs well into the second half of the estrous period. There was a highly significant breed difference in the time of ovulation, which was positively correlated with the duration of estrus. Thus, the mean durations of estrus and the interval between the onset of estrus and ovulation for the Large Black and Large White breeds were 62.5 and 42-54 hours, 47.6 and 18-36 hours, respectively. It was calculated that ovulation was delayed 6 hours for each 7.8-hour extension of estrus.

Burger attempted to determine the length of time required for all

of the follicles of an individual sow to rupture. Although the results were rather inconclusive, he estimated that complete ovulation of all definitive follicles required approximately 6.5 hours.

2. Rate of Ovulation

The ovulation rate of the sow at each estrus has been reported to average 16.4 ova, with a range of 10 to 25 (39). Statistically, the left ovary may contribute more ova at each ovulation than the right ovary (45, 63). Burger (11) and Self *et al.* (49) reported significant differences in ovulation rate with breed, and Squiers *et al.* (55) found that inbreeding may reduce the number of ova shed. In addition to these genetic effects, the most important factors contributing to the variability in ovulation rate are sexual age and the nutritional status immediately preceding and during the breeding season.

In general, advancing sexual age increases ovulation rate. Robertson *et al.* (45) determined the number of ova shed at two consecutive estrous periods by counting the number of regressing and newly formed corpora lutea in the ovaries of Chester White and Poland China gilts slaughtered immediately following the second estrous period after puberty. They observed a highly significant increase of ovulation rate at succeeding estrous cycles for both breeds. The average increase in the number of ova shed per animal for each breed was 1.6 and 1.2, respectively. Age at puberty was not associated with ovulation rate, and within this limited age difference calendar age was not as important as sexual age in determining ovulation rate. Warnick *et al.* (62) also presented data suggesting an increase of ovulation rate with increasing sexual age through the third estrus after puberty, and Burger (11) found that the average number of ova shed by 7- to 8-month-old gilts was 14.12, while 15- to 17-month-old sows had an ovulation rate of 16.17 ova. Squiers *et al.* (55) compared the ovulation rate of 277 gilts averaging 255 days of age with that of 72 older sows. The average numbers of ova shed by the gilts and sows were 11.5 ± 2.53 and 15.4 ± 3.40 , respectively. They estimated that in the gilt each 10-day increase of age at conception resulted in an increase in ovulation rate of 0.35 ova. The average age at conception was 226 ± 23.2 days. Zimmerman *et al.* (71) reported a substantial increase in ovulation rate at second estrus over the previous one in Chester White and crossbred gilts.

The practice of "flushing," or of raising the nutrient intake of sows before the breeding season in order to increase the number of pigs farrowed, has been recommended by animal husbandmen for a number of years (50). Recently, several investigators have studied, in some

detail, the influence of food intake on ovulation rate Zimmerman *et al* (71) limited the prepuberal nutrient intake of 32 crossbred and 16 Chester White gilts by feeding a high-fiber ration (13.3% fiber). At puberty, the gilts were divided randomly into four groups. One group was self-fed the same high-fiber ration, while the other three groups were self-fed a low-fiber, fattening ration (5.9% fiber) beginning on either the 8th, 12th, or 16th days of the first estrous cycle. All gilts were slaughtered 3 to 5 days after the onset of the second estrous period. In all cases the ovulation rate was greater at second than at first estrus. However, the increase was significantly greater in the gilts receiving the fattening ration from the 12th and 16th days of the first estrous cycle in comparison with the nonflushed controls. The increase in ovulation rate was independent of such individual characteristics as the pre- and postflush body weights, degree of finish, average daily gain, and food consumption during the flushing period. In an earlier paper (49), this same group of investigators compared the effects of self-feeding with limited feeding, at two-thirds the self-fed rate, upon the ovulation rates of Chester White and Poland China gilts in each of two years. The experimental rations were fed from about the 70th day of age until the 25th day after the onset of the second estrous period, when the animals were slaughtered. Mating occurred at the second estrus. Some of the gilts were full fed and others were limited-fed throughout the experimental period. In addition, some were full fed until puberty and during the first estrous cycle and then were limited-fed during the first 25 days of gestation, others were limited-fed except during the first estrous cycle, others were full fed only until puberty, while others were limited fed only until puberty. During the first year, the gilts full fed throughout the experimental period had a higher ovulation rate than either those fed at two-thirds the self-fed rate throughout the entire experimental period or those receiving the limited ration only after the attainment of puberty. However, in comparison with full-feeding, limited-feeding during only the prepuberal stage of sexual development did not reduce the ovulation rate. During the second year, gilts full-fed from 70 days of age through the first estrous cycle ovulated at a higher rate than those which were limited-fed throughout the experimental period, but they did not differ from those full fed only during the first estrous cycle, indicating that only a short period of full-feeding is necessary to stimulate the maximum rate of ovulation. The number of ova shed at the second estrus was positively correlated with the gain in body weight during the first estrous cycle.

In contrast to the favorable effects of "flushing" which involves an

increase in the consumption of total energy and possibly protein, nutritional inadequacies may reduce the ovulation rate. For example, Johnsen *et al.* (32) found that a ratinn deficient in vitamin B₁₂ reduced the ovulation rate of gilts. Teague (59) published data suggesting the existence in ground, sun-cured alfalfa of an unidentified factor which increased the number of ova shed. Gilts fed a ration containing 18% alfalfa meal had an average ovulation rate of 13.5 ± 1.51 in comparison with a rate of 11.9 ± 2.0 in control gilts fed a presumably adequate ration devoid of alfalfa meal.

E. Experimental Modification of the Estrous Cycle

1. Use of Gonadotropins

The use of various gonadotropic and ovarian hormones to induce or to inhibit estrus and ovulation, either in the normal-cycle sow or during lactational diestrus, has been investigated for a number of years. Although quite variable, the results of these studies furnish some insight into the effectiveness of such hormones in regulating the reproductive cycle.

The early investigations of Faiermark (25) and Hvaton *et al.* (31) indicated that various gonadotropins would induce both estrus and ovulation, if given in proper amounts to the sow in cycle. Only those materials containing appreciable quantities of follicle-stimulating activity were effective. In a more definitive study, Tanabe *et al.* (58) found that the administration of equine gonadotropin during the luteal phase of the cycle (5 days after estrus) would not produce heat or ovulation, although a number of large ovarian follicles did develop. Equine gonadotropin, administered during the luteal phase of the cycle and followed 5 days later by the injection of unfractionated sheep pituitary extract, induced estrus and ovulation with an average of 13.1 ova shed. When the sows were bred, none of the 97 ova recovered from the reproductive tracts was fertile, although two contained sperm in the zona. It is not clear whether the ova were nonviable or whether the spermatozoa failed, in most cases, to reach the ova. Five of 9 sows receiving equine gonadotropin during the follicular phase of the cycle (16th day after estrus) exhibited estrus within 48 to 56 hours. Estrus and ovulation were induced by injecting pituitary extract 5 days after the administration of equine gonadotropin during the follicular phase of the cycle. The average number of corpora lutea formed was 25.3 and an average of 5.3 fertile ova were recovered from each sow. Ovulation occurred 36 to 48 hours after the intravenous administration of sheep pituitary extract alone on either the 6th, 17th, or 20th days

of the cycle, although the ovulation rate was higher when the extract was injected during the follicular phase. Du Mesnil du Buisson (23) also reported the successful induction of ovulation in gilts with sheep pituitary extract. Ovulation occurred about 38 hours after treatment and the ova remained viable for less than 4 hours, a finding which may explain the poor fertility of ova ovulated during the luteal phase of the cycle. Day *et al* (22) found that estrus and ovulation were induced in gilts by the administration of "pure" FSH (Armour preparation) followed in 24 hours by estradiol during the follicular, but not during the luteal, phase of the estrous cycle.

The successful induction of estrus, ovulation, and subsequent fertilization in the sow would have considerable practical application, since it could increase reproductive capacity and the number of pigs farrowed. Cole and Hughes (14) and Heitman and Cole (28) investigated the use of equine gonadotropin with this in mind. In general, the administration of this hormone early in lactation (before the 40th day) was not as effective as injections made late in the lactation period. In the latter study, 76 and 86% of the sows receiving the hormone between the 20th and 39th days and between the 40th and 50th days, respectively, exhibited estrus. Following breeding, 44% of the 20- to 39-day group and 66% of the 40- to 50 day groups farrowed. Estrus usually occurred between 4 and 6 days after hormone administration. The results were not influenced by the number of suckling young. Allen *et al* (2) reported that only 1 of 20 control sows, while 3 of 18, and 17 of 26 sows receiving 1,000 IU of gonadotropin on the 20th and 40th days of lactation, respectively, exhibited estrus during the 56-day lactation period.

2 Use of Gonadal Hormones

The gonadal hormones presumably effect the estrous cycle by causing inhibition of pituitary gonadotropin production or release. Ulberg *et al* (60) studied the use of progesterone to control the estrous cycle of the normally cycling sow. Daily injections of 25, 50, or 100 mg per animal effectively inhibit estrus if administered sufficiently early in the cycle (15th day), but the results are quite variable if the injections are initiated on the 19th day of the cycle. Doses less than 25 mg daily are ineffective, irrespective of the time of administration. The sows exhibited heat 6 to 7 days after withdrawal of the high levels of progesterone (100 mg daily), and ovulation appeared to be normal, although no information on fertilization was obtained. The ovaries of a high percentage of the gilts receiving the intermediate dosage of pro

gesterone (50 mg.) contained cystic follicles. These animals did not exhibit estrus over the 7- to 26-day postprogesterone-treatment period. Observing that a moderate dose of progesterone results in cystic follicles which do not ovulate, while the higher dose does not prevent ovulation after it is withdrawn, the investigators postulated that the latter produces absolute inhibition of pituitary gonadotropic production, while the moderate dose is sufficient to depress LH but not FSH, resulting in an imbalance of gonadal regulation. In a reinvestigation of this study (5), results were much more variable. Only 1 of 9 gilts receiving 25 mg. of progesterone daily exhibited estrus after withdrawal, and 7 of the 9 possessed cystic follicles and had not ovulated. One group of gilts receiving 100 mg. of progesterone daily, starting on the 15th day of the cycle, did not develop cystic follicles and reacted similarly to those reported in the earlier study, but in two other groups, receiving the same amount of hormone starting on either the 10th or 15th days of the cycle, the hormone increased the number of follicular cysts. In general, however, the results show that the injection of 100 mg. of progesterone daily, starting early in the follicular phase of the cycle, will inhibit the estrous cycle. After injections are stopped, the cycle is resumed with some modifications from normal. Subsequent to hormone treatment the estrous period is shortened, the incidence of follicular cysts increases, and fertility, as measured by the number of fertile ova recovered, is reduced. The investigators again advanced the theory that the development of follicular cysts is due to a differential inhibition of the pituitary by moderate doses of progesterone, resulting in an overproduction of FSH in proportion to LH. The variable results obtained with a daily dose of 100 mg. of progesterone, however, indicate that this is not the optimum level to obtain complete pituitary inhibition.

The various substances possessing estrogenic properties have variable effects on the estrous cycle, depending, for the most part, upon the time of administration in relation to estrus and upon the amount employed. In Europe, a number of investigators have used relatively high levels of these substances to inhibit the estrous cycle, and in general, the cycle has been suppressed for several months, particularly when treatment was initiated during the luteal phase of the cycle (4, 8, 37, 53, 54, 56, 70). At these levels, a number of substances having estrogenic activity were effective, and older sows were more sensitive than were gilts (53). Moderate amounts of estrogen did not inhibit the onset of sexual maturity (54), but higher levels delayed puberty (56) when injected into sexually immature gilts. Kidder *et al.* (34), in a more recent study, investigated the effects of injecting relatively small amounts (3

mg) of diethylstilbestrol into gilts on either the 6th, 11th, or 16th day of the estrous cycle. The results differed with the time of administration. Administration on the 6th day of the cycle had no effect, but the cycle was prolonged for about 6 days in the gilts injected on the 11th day of the cycle. When the latter were bred at estrus, fertile ova were recovered from the reproductive tracts. Ovulation appeared to have been relatively normal, although the ovaries of all gilts examined contained abnormally large luteinized follicles. The effects of hormone injection on the 16th day of the cycle were quite variable. In some gilts the cycles were shortened, and in others greatly prolonged. In the latter, normally appearing corpora lutea were present in the ovaries and the endometria were extremely edematous.

The relatively high relaxin content of the corpora lutea of the sow suggests that this hormone might be released during the luteal phase of the cycle. Zarrow *et al* (69) found the diameter of the cervix to be smaller in castrate as compared with normal gilts. Relaxin administration following a priming dose of estrogen caused significant dilation and associated increase in water content and depolymerization of the ground substance of the uterine cervix.

REFERENCES

- 1 Adams E, *Endocrinology* 26 891 (1940)
- 2 Allen, A D, Lasley, J F and Uren, A W, *J Animal Sci* 16, 1097 (1957)
- 3 Altmann, M, *Am J Physiol* 126, 421 (1939)
- 4 Brzez E, *Wien tierarztl Monatsschr* 37, 197 (1950), *Animal Breeding Abstr* 20, 161 (1952)
- 5 Baker L N, Ulberg L C, Crummer, R H, and Casida, L E, *J Animal Sci* 13 648 (1954)
- 6 Baker, L N, Wochling H L, Casida, L E, and Crummer, R H, *J Animal Sci* 12 33 (1953)
- 7 Barker, W L, *Endocrinology* 48, 772 (1951)
- 8 Benesch, F, *Wien tierarztl Monatsschr* 39, 393 (1952), *Animal Breeding Abstr* 21, 283 (1953)
- 9 Bloor, W R, Okey, R, and Corner, C W, *J Biol Chem* 86, 291 (1930)
- 10 Boyd E M, and Elden, C A, *Endocrinology* 19, 599 (1935)
- 11 Burger, J F, *Onderstepoort J Vet Research* 25, Suppl 2, 218 (1952)
- 12 Casida L E, *Anat Record* 61, 389 (1935)
- 13 Cleveland R, and Wolfe J M, *Am J Anat* 53, 191 (1933)
- 14 Cole, H H, and Hughes E H, *J Animal Sci* 5, 25 (1916)
- 15 Corner, C W, *Am J Anat* 26 117 (1919)
- 16 Corner, C W, *Carnegie Inst Wash Contribs to Embryol* 29, 65 (1920)
- 17 Corner, C W, *Carnegie Inst Wash Contribs to Embryol* 13 117 (1921)
- 18 Corner, C W, *Am J Anat* 32, 345 (1923)
- 19 Corner, C W, and Amshuegh A E, *Anat Record* 12 287 (1917)
- 20 Casida, A, and Corner, C W, *Endocrinology* 49, 340 (1951)
- 21 Curtis, Q F, *Proc Soc Exptl Biol Med* 35, 560 (1937)

2. Day, B. N., Hazel, L. N., Anderson, L. L., and Melampy, R. M., *J. Animal Sci.* 16, 1104 (1957).
23. Du Mesnil du Buisson, F., *Ann. endocrinol. (Paris)* 15, 333 (1954); *Biol. Abstr.* 31, 1690 (1957).
24. Elden, C. A., *Proc. Soc. Exptl. Biol. Med.* 32, 515 (1934).
25. Faiermark, S. E., *Problemy Zootech. Eksptl. Endokrinol.* 2, 78 (1935); *Animal Breeding Abstr.* 4, 63 (1936).
26. Faiermark, S. E., and Singerman, L. S., *Bull. biol. méd. exptl. U.R.S.S.* 6, 89 (1938); *Animal Breeding Abstr.* 7, 233 (1939).
27. Foote, W. C., Waldorf, D. P., Chapman, A. B., Self, H. L., Grummer, R. H., and Casida, L. E., *J. Animal Sci.* 15, 959 (1956).
28. Heitman, H., Jr., and Cole, H. H., *J. Animal Sci.* 15, 970 (1956).
29. Hisaw, F. L., and Zarrow, M. X., *Proc. Soc. Exptl. Biol. Med.* 69, 395 (1948).
30. Hollandbeck, R., Baker, B., Jr., Norton, H. W., and Nalbandov, A. V., *J. Animal Sci.* 15, 418 (1956).
31. Ilyatov, B. P., Bogdanova, M. P., and Kuznecov, N. N., *Problemy Zootech. Eksptl. Endokrinol.* 2, 98 (1935); *Animal Breeding Abstr.* 4, 64 (1936).
32. Johnsen, H. H. K., Moustgaard, J., and Olsen, N. H., *Dansk Maanedsskrift Dyrlaeger* 63, 1 (1952); *Animal Breeding Abstr.* 23, 172 (1955).
33. Keye, J. D., *Bull. Johns Hopkins Hosp.* 34, 60 (1923).
34. Kidder, H. E., Casida, L. E., and Crummer, R. H., *J. Animal Sci.* 14, 470 (1955).
35. Kimura, C., and Cornwell, W. S., *Am. J. Physiol.* 123, 471 (1938).
36. King, J. L., *Am. J. Physiol.* 81, 725 (1927).
37. Kment, A., and Halama, A., *Wien. tierärztl. Monatsschr.* 39, 521 (1952); *Animal Breeding Abstr.* 21, 285 (1953).
38. Krallinger, H. F., *Wiss. Arch. Landwirtschaft., Abt. B, Arch. Tierernähr. u. Tierzucht* 8, 436 (1932).
39. Lasley, E. L., *J. Animal Sci.* 16, 335 (1957).
40. Lewis, L. L., *Oklahoma Agr. Expt. Sta. Bull.* 96, 47 pp. (1911).
41. McKenzie, F. F., *Missouri Agr. Expt. Sta. Research Bull.* 86, 41 pp. (1926).
42. McKenzie, F. F., and Miller, J. C., *Missouri Agr. Expt. Sta. Bull.* 285, 43 (1930).
43. Phillips, R. W., and Zeller, J. H., *Anat. Record* 85, 387 (1943).
44. Pomeroy, R. W., *J. Agr. Sci.* 45, 327 (1955).
45. Robertson, G. L., Grummer, R. H., Casida, L. E., and Chapman, A. B., *J. Animal Sci.* 10, 647 (1951).
46. Robinson, G. E., Jr., and Nalbandov, A. V., *J. Animal Sci.* 10, 469 (1951).
47. Schmidt, K., and Bretschneider, W., *Tierzucht* 8, 119 (1954); *Animal Breeding Abstr.* 22, 232 (1954).
48. Seckinger, D. L., *Bull. Johns Hopkins Hosp.* 34, 236 (1923).
49. Self, H. L., Grummer, R. H., and Casida, L. E., *J. Animal Sci.* 14, 573 (1955).
50. Smith, W. W., "Pork Production." Macmillan, New York, 1937.
51. Snyder, F. F., and Corner, G. W., *Am. J. Obstet. Gynecol.* 3, 358 (1922).
52. Spalding, J. F., Berry, R. O., and Moffit, J. C., *J. Animal Sci.* 14, 609 (1955).
53. Spörri, H., and Candinas, L., *Schweiz. Arch. Tierheilk.* 93, 129 (1951); *Animal Breeding Abstr.* 19, 225 (1951).
54. Spörri, H., and Candinas, L., *Experientia* 7, 267 (1951); *Animal Breeding Abstr.* 19, 498 (1951).
55. Squiers, G. D., Dickerson, G. E., and Mayer, D. T., *Missouri Agr. Exptl. Sta. Research Bull.* 494, 40 (1952).

- 56 Stift, K, *Wien tierarztl Monatssehr* 38, 168 (1951), *Animal Breeding Abstr* 21, 66 (1953)
- 57 Struve, J, *Fuhling's Landwirtsch Ztg* 60, 832 (1911), cited by Corner, see reference 17
- 58 Tanabe, T Y, Warnick A C, Casida, L E, and Crummer, R H, *J Animal Sci* 8 550 (1949)
- 59 Teague, H S, *J Animal Sci* 14 621 (1955)
- 60 Ulberg, L C, Crummer, R H, and Casida, L E, *J Animal Sci* 10, 665 (1951)
- 61 Warnick, A C, Casida, L E, and Crummer, R H, *J Animal Sci* 9, 66 (1950)
- 62 Warnick, A C, Wiggins, E L, Casida, L E, Crummer, R H, and Chipman, A B *J Animal Sci* 10, 479 (1951)
- 63 Warwick B L, *Anat Record* 33 29 (1926)
- 64 Wetli, W, *Dissertation, Veterinar medizinische Fakultät der Universität Zurich* 42 pp (1942), *Animal Breeding Abstr* 12, 35 (1944)
- 65 Whitelaw, M J, *Am J Obstet Gynecol* 25, 475 (1933)
- 66 Wiggins, E L, Casida, L E, and Crummer, R H, *J Animal Sci* 9, 277 (1950)
- 67 Wilson K M, *Am J Anat* 37, 417 (1926)
- 68 Wolfe, J M, *Am J Anat* 48 391 (1931)
- 69 Zarrow, M X, Neher, C M, Sikes, D, Brennan, D M, and Bullard, J F, *Am J Obstet Gynecol* 72 260 (1956)
- 70 Zehetner, F, *Wien tierarztl Monatssehr* 39, 526 (1952), *Animal Breeding Abstr* 21, 285 (1953)
- 71 Zimmermann, D R, Self, H L, and Casida, L E, *J Animal Sci* 16, 1099 (1957)

CHAPTER II

The Estrous Cycle of the Dog

A. C. ANDERSEN AND ELOISE WOOTEN

	Page
I. Introduction	359
II. Development of the Genital Organs to Puberty	360
III. The Estrous Cycle	364
A. Duration and Interval	364
B. Manifestation of Estrus	365
1. Symptoms and Sexual Behavior	365
2. Peripheral Blood Values	366
C. Anatomy of the Genital Organs—Gross and Histological Changes	367
1. Anestrus	367
2. The Estrous Cycle	371
3. Vaginal Smear	379
D. Clinical Aspects of Pseudopregnancy	381
IV. Factors Influencing the Estrous Cycle	384
A. Environment	384
1. Season	384
2. Nutrition	384
3. Ecology	385
4. X-Irradiation	385
B. Physiology	385
1. Hypophysectomy, Ovariectomy, Adrenalectomy	386
2. Replacement Therapy	387
3. Hormone Injection in the Intact Animal	388
C. Pathology	390
V. Breeding, Whelping, Lactation, and Puppy Production	392
References	393

I. INTRODUCTION

The domestic dog (*Canis familiaris*) is represented by a large number of breeds. Through centuries of selective breeding, this species has been developed to perform a wide variety of tasks on behalf of mankind. Yet, popular concepts concerning the breeding habits of the dog remain obscured by superstitions. It is only through a basic understanding of the changes occurring during the estrous cycle that problems related to reproduction can be properly evaluated. For this reason, reproduction is discussed throughout the dog's life span, i.e., phases of growth, maturity, and senility. Material obtained from observations on some 400 female beagles maintained for life span studies are included in the text (7).

The female dog is monestrous in that several months of sexual inactivity occur between breeding periods. In 1900, Hcape (51) proposed terms to designate phases of the estrous cycle which have since been widely adopted. He designated proestrus as the beginning of the sexual season or "coming into heat"; estrus as the period of sexual desire; metestrus, a gradual subsiding of sexual activity; and anestrus, a period of rest or quiescence. It might be mentioned that the use of the word "heat" stems from the elevated temperature felt upon palpation of the external genitalia during the period of sexual activity. In discussing the estrous cycle in the dog, "diestrus" should not be used, since this term refers to the period of quiescence in polyestrous animals with shorter cycles.

II. DEVELOPMENT OF THE GENITAL ORGANS TO PUBERTY

This account is intended primarily to provide the reader with a brief timetable concerning development of the reproductive system in the dog. In contrast to other animals, the dog exhibits considerable individual variation between the time of ovulation and implantation; therefore, a wide divergence in size and development between litters is encountered when fetal age is calculated from the time of mating. Where possible, crown-rump (C-R) measurements and body weights have been included for the sake of comparison.

In the dog, between 5 and 10 days are required for fertilized ova to reach the anterior horn of the uterus; the embryo, at this time, has reached the 16-32-cell stage (9). Fetal implantation occurs 18 to 20 days after conception (1). At the beginning of the second trimester of gestation, the zonular placenta has been established and fetal development proceeds at a rapid pace.

As shown in Fig. 1, the 23-day embryo (C-R, 5.0 mm.) has completed delimitation. At this stage, potential gonadal tissue is represented by unorganized somatic and germ elements. In embryos of 7.5 mm., the urogenital ridges are well-defined, and by the 28th day, indifferent gonads project from the ventromedian surfaces (Fig. 2). The gonads, at this time (C-R, 15.0 mm.), consist of undifferentiated cells. According to Jonckheere (58), in the 30-day fetus, the initial wave of cells from the germinal epithelium are penetrating into the gonad to form the medulla. These cells soon form a core within the gonad separated from the germinal epithelium by the primitive tunica albuginea. Further development of the gonad distinguishes the sexes. Differentiating seminiferous tubules can be recognized in gonads destined to become testes before oögonia appear in the developing cortex of the ovary (35-day

fetus, C-R, 65 mm.; wt., 25 g.). Clusters of oocytes appear immediately below the germinal epithelium in the 43-day fetus (C-R, 109 mm.; wt., 88.4 g.). Cortical proliferation and differentiation continue, and the ovary at birth may contain an estimated 700,000 follicles (92).

As ovarian development continues, the fetal reproductive system takes

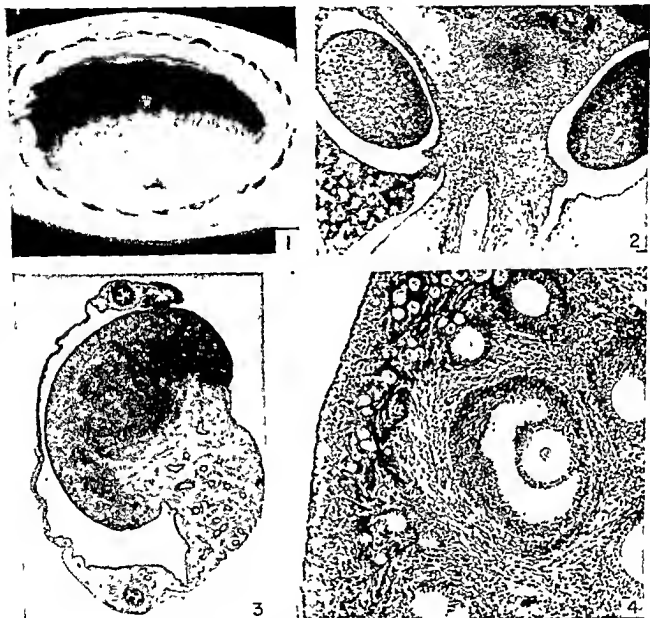


FIG. 1. Twenty-three day beagle fetus (5 mm.) in utero. At this stage, gonads are represented by unorganized somatic and germ elements.

FIG. 2. Indifferent gonads in 28-day fetus (15 mm.). Magnification: $\times 29$.

FIG. 3. Ovary from beagle pup at birth. Note the relatively small cortical rim of follicles as compared to the large core of medullary elements. The bursal sheath extends from the broad stalk and shows 2 cross sections of the oviduct and the fimbria on the free margin. Bouin's, paraffin, hematoxylin, and eosin. Magnification: $\times 6$.

FIG. 4. Cortical zone of ovary from 6½-month-old beagle showing various size follicles. Magnification: $\times 28$.

In placental mammals, the paired Müllerian ducts fuse posteriorly in the body of the uterus and vagina; these portions are separated by a thickening, the cervix (81). Anteriorly, the Müllerian ducts develop into the bicornate horns of the uterus. From each uterine horn, a slender tubule (the oviduct) continues across the ventral surface of the ovary. Posteriorly, the vagina empties into the vestibule or urogenital sinus, a common passageway for both the genital tract and urinary system. External genitalia are visible in the 35-day fetus (C-R, 65 mm.).

At birth, the ovary is globular in shape and in the beagle measures about 4.0×6.0 mm. Cortical and medullary elements (Fig. 3) are represented approximately in the ratio of 1:5. The cortical zone, covered by cuboidal epithelium, contain clusters of oöcytes and primordial follicles; the latter are surrounded by delicate connective tissue elements and capillaries. Deeper within the ovary, epithelial cells intermingle with connective tissue, blood vessels, and lymphatics. The ovigerous cortex and stromal elements of the medulla contrast sharply with the loose connective tissue of the hilum, which continues as the mesovarium or ovarian stalk (Fig. 3). The mesovarium contains blood and lymphatic vessels and vestiges of the provisional male duct system (epoöphoron). As shown in Fig. 2, a thin connective tissue sheath extends from the mesovarium over the surface of the ovary to form the ovarian bursa. This photomicrograph also shows the fimbria on the free margin of the sheath as well as two cross sections of the oviduct.

Figure 5 illustrates the growth curve in the beagle breed from birth to puberty. Puberty occurs only after the growth plateau is attained. During the period of rapid growth, ovarian follicles gradually increase in size. True primary follicles are found in the ovary when the puppy is 15 days old (84); by the 5th month, the cortex contains follicles which show evidence of liquor formation (56). Graafian follicles are found at the corticomedullary junction when the puppy is $6\frac{1}{2}$ months old (Fig. 4). Until 8 months, when the growth plateau is reached, the vast majority of follicles undergo degeneration. At 8 months, maturing Graafian follicles are present in the ovary, and external signs of puberty may be manifested. However, both individual and breed differences exist with respect to the time of puberty. While larger breeds may not manifest estrus until $1\frac{1}{2}$ to 2 years of age (47), beagles generally show signs of estrus between 10 and 14 months. Once definite signs of estrus are apparent, a rhythmic cycle of potential reproductive ability continues throughout the life span of the dog.

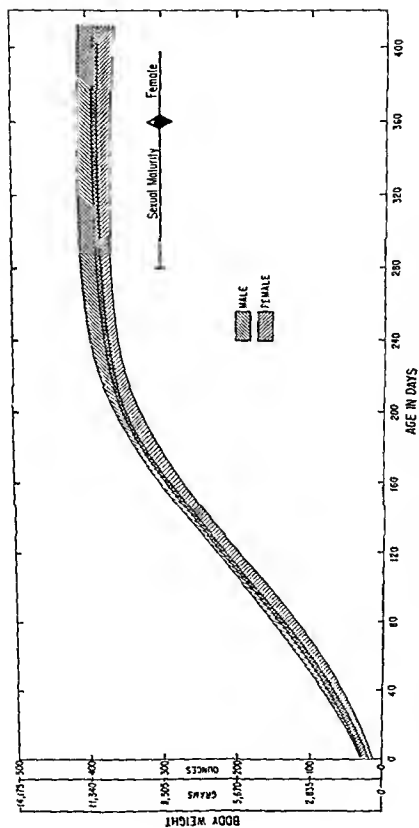


FIG. 5. Growth curve in the beagle from birth to puberty.

III. THE ESTROUS CYCLE

A. Duration and Interval

In discussing the duration of estrus, Heape (51) stated: "In the bitch it lasts a variable time, variable both in different individuals of the same species and in the same individuals at different times." This has been confirmed by several authors (11, 45, 69, 78, 107). The results of all of these investigators are within the time limits for phases of the cycle proposed by Evans and Cole (37), i.e., proestrus—9 days; estrus—9 days; metestrus—80 to 90 days. Normal variations expected in these periods are 3 to 16 days in proestrus; 4 to 12 days in estrus; and up to 90 days in metestrus. At puberty, estrus may be somewhat longer (75). Once an individual bitch has established an interval, each succeeding proestrus, estrus, and metestrus phase of the cycle shows little variation. The length of these periods appears to be independent of body size (107).

It has generally been accepted that the bitch has two estrous periods at 6-month intervals. The impression has been that little variation in the cycle exists. However, one rarely finds a bitch coming into season on the same calendar date from year to year. Williams (47a) stated: "I have always regarded the interval between cycles as extremely variable and I think it might be profitable to sidestep this by abandoning the conception that a six-monthly cycle is normal." *The interval between estrous cycles is largely determined by the length of anestrus.* In contrast to proestrus, estrus, and metestrus, the length of anestrus seems to vary with size; smaller breeds may have a 4-month cycle while larger breeds (Great Dane) may have an 8-month cycle (69). Most females show a slight but progressive increase in the intervals between estrus until 4 years of age. The aged female commonly has irregular estrous cycles and often an extended period of anestrus (2, 82).

Rowlands (91) maintained records from 50 females over a 4-year period and found the estrous interval to be 7.7 months. Our kennel records over a 5-year period showed a 7.3-month interval in virgin beagles and a 7.0-month interval in bred dogs, as shown in Table I.

TABLE I
THE ESTROUS CYCLE IN THE BEAGLE

	Nonbred	Bred
Number of dogs	25	29
Age at first estrus (days)	352	348
Estrous periods (total)	143	164
Estrous cycles per dog	5.72	5.65
Length of cycle (days)	228 (SD 70)	212 (SD 56)

The criteria used to obtain these data were vulva palpation and a sanguineous discharge. This method proved 80.5% effective in detecting estrus by the acceptance test in the bred group. It will be noted that nonbred dogs showed a slightly longer interval between estrous cycles (16 days) with a significantly larger standard deviation (SD) than found in the bred group. This verifies the clinical observation that irregular estrous periods are more common among nonbred dogs. It would seem that the average estrous interval is approximately 7 months, although considerable individual and breed variations exist.

B Manifestation of Estrus

1 Symptoms and Sexual Behavior

Female dogs experiencing a normal estrous rhythm usually exhibit some signs several weeks before coming into heat, in general, their appetite and appearance improve. If given the opportunity, they prefer to associate with male dogs. Some females may actually resent the companionship of other females when male dogs are in the vicinity, this resentment is especially noticeable toward spayed females.

A few days prior to visible signs of proestrus, most female dogs become listless and somewhat indifferent. On occasion, the maiden female may refuse food. Excitement, tremors, and tetanic spasms in a 5 year-old terrier coming into heat were reported by Moss (74). A few cases have been observed in which convulsions preceded signs of proestrus. These seizures are without any apparent cause and stop as soon as external signs of proestrus become evident.

Proestrus is characterized by swelling of the external genitalia, and by a sanguineous discharge from the vulva which follows within 2 to 4 days. As the flow of blood-tinged fluid increases the vulva and vestibule become enlarged, and upon palpation feel turgid to the touch. The disposition of the female changes, she becomes restless, excitable, and does not respond to commands which at other times are immediately obeyed. She drinks increasing quantities of water and urinates frequently, which apparently attracts male dogs. If not restrained, she will roam to lure male dogs but will resist copulation. The higher mortality rate observed in dogs under field conditions as compared to those in a kennel is in part attributed to the tendency to roam during proestrus and estrus (4). The attracting factor in urine from female dogs has not been identified. Berel and Gilmore (16) demonstrated that the bitch is not the attracting factor, since urine placed in cardboard containers aroused sexual libido in male dogs. If the female is fed

sufficient amounts of chlorophyll (8.0 mg. per kg.), she will attract fewer males, but breeding is not jeopardized (43).

There is no method of determining when a female dog enters estrus other than by acceptance. Most bitches will accept the male within 6 to 12 days from the onset of proestrus. At this time, the bloody discharge is greatly reduced, and she stands for coitus. She assumes a position with an elevated tail-head and the tail held horizontally to one side. The inexperienced bitch will often assume the breeding stance several times between short periods of play before allowing copulation, while experienced females usually accept the male without undue play or teasing.

Among "proud" bitches, there is a tendency to select the male; this is especially noticeable in the Saluki and Labrador breeds (10). Sexual libido on the part of the male may be a factor. For instance, young males may breed a willing female sooner than an older male (109). Some females may assume the breeding stance but not allow copulation until the swelling and sensitivity of the genital tract has receded.

After estrus the external genitalia become flabby, and a scant dark brown discharge is evident. The female becomes increasingly tranquil and docile; outwardly, she may appear somewhat unthrifty. Within 2 to 6 weeks, a small amount of whitish or opaque discharge appears at the ventral commissure of the vulva which gradually disappears as the external genitalia returns to normal. Then, the female once again conditions herself for the next estrous period.

Upon occasion, a female may be seen mounting dogs of either sex, exhibiting pelvic thrusts for a variable length of time. Such action is most commonly observed among dogs in heat, but some may show this behavior during any phase of the estrous cycle. Walton (106) feels that "riding" in the dog is neither cyclic nor indicative of sexual receptivity. Excessive sexual desire (nymphomania), in female dogs, on the other hand, is generally associated with the pathological condition of cystic ovaries (87).

2. *Peripheral Blood Values*

Changes in the physiological state of the animal during the estrous cycle might be expected to produce variations in peripheral blood values. In our laboratory, an attempt was made to determine if such variations occurred. Nonbred and bred groups of mature female beagles were tested for estrus by physical examinations and by testing with males. Blood samples were obtained from each group at weekly intervals throughout the estrous cycle. The changes in blood values were more

pronounced in the bred group, similar but less pronounced trends were observed in the nonbred group (6).

No significant variation in leucocytic elements was noted during the infertile cycle, although bred dogs commonly reveal a 35–40% increase in leucocytes during late gestation (6). During proestrus, hematocrit values increased 10–12% above those established for beagles in anestrus (5). The packed cell volume increased from 46.6 to 57.5 ml./100 ml. blood (SD, 2.5) by late proestrus and early estrus. Correspondingly high erythrocyte and hemoglobin values were observed, namely, 8.49×10^6 (SD, 0.31) and 19.0 (SD, 0.54), respectively. During metestrus, erythrocytic elements gradually decrease to values found at anestrus. Further blood variations observed in this experiment were limited to an increase in sedimentation rate. A significant rise, 5.5 mm. per hr. (SD, 7.4), in sedimentation rate was not apparent until the 6th to 7th week of metestrus. The skewed value indicates that, at this time, the sedimentation rate was between 5–15 mm. In contrast, pregnant dogs commonly show a mean sedimentation rate of 30 mm. in the 6th to 7th week of gestation (6). Further study to confirm the results of this experiment is required.

C. *Anatomy of the Genital Organs—Gross and Histological Changes*

Anatomically, the genital organs include the paired ovaries, oviducts, uterine cornu, uterine body, cervix, vagina, and vestibule (Fig. 6). With the advent of puberty, ovarian activity under the influence of gonadotropins is responsible for the cyclic changes exhibited by all other portions of the genital tract. The release of estrogen and progesterone, in turn, from the activated ovary produces grossly observable and extensive microscopic alterations. To fully appreciate these changes, it is necessary to explore the anatomy of the tract prior to the advent of estrus.

1. *Anestrus*

That portion of the genital tract located within the abdominal cavity is suspended by two peritoneal folds, the broad ligaments. These ligaments extend from the dorsolateral peritoneal cavity to the uterus, oviducts, and ovaries as the mesometrium, mesosalpinx, and mesovarium, respectively. Posteriorly, the ligaments merge into the retroperitoneal connective tissue within the pelvic canal. Anteriorly, the broad ligaments are attached to the dorsolateral abdominal wall in their passage toward the kidneys to which they adhere. Blood vessels, lymphatics, and nerves, interspersed with lobules of adipose tissue, are particularly

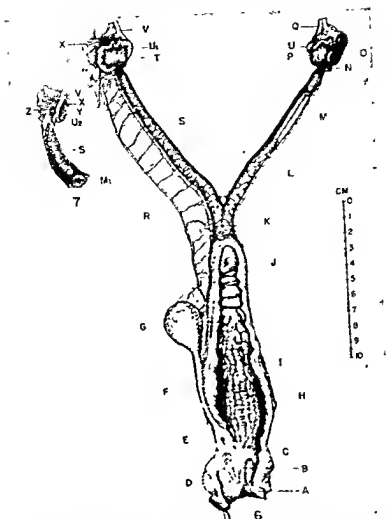


FIG. 6. Ventral view of genital tract at first estrous period from beagle (4A7) sacrificed and dissected 18 hours after first acceptance; 382 days of age, 7.4 kg; measured 31 cm. in height and 42 cm. in length.

KEY: A, Vulva. B, Vestibule. C, Hymen. D, Vestibular bulb. E, Urethral orifice. F, Ureter. G, Bladder. H, Vaginal mucosa with longitudinal folds and rugae. I, Wall of vagina, reflected to expose lumen. J, External os of cervix protruding from dorsal wall of vagina. K, Body of the uterus. L, Round ligament of uterus. M, Uterine cornu revealing small hymen. N, Distal portion of oviduct. O, Cut surface of ovary showing developing corpora lutea. P, Ovarian bursa. Q, Portion of ovarian bursa joining ovarian ligament. R, Broad ligament containing congested uterine blood vessels. S, Uterine cornu. T, Ventral surface of ovarian bursa almost devoid of fat as compared to lobules of adipose tissue in surrounding area. U, Oviduct transverse ventral surface of ovarian bursa. U₁, Distal portion of oviduct passing along medial surface of ovary. V, Ovarian ligament. X, Capillaries within ovarian bursa. (Drawn by A. C. Andersen.)

FIG. 7. Dorsal surface of right ovary, bursa, and anterior uterine cornu at first estrous period.

KEY: M₁, Cross section of uterine cornu. U₂, Oviduct passing into fimbriae. V, Ovarian ligament. X, Capillaries within ovarian bursa. Y, Margo ovaricus. Z, Fimbriated mucosa (rütteliche Masse) viewed through the ovarian slit. (Drawn by A. C. Andersen.)

noticeable in the mesometrium. Blood vessels anastomose as the mesometrium incases the uteri, forming the outer, flattened, serosal layer. The mesovarium envelops the ovary, forming the ovarian bursa. The oviduct passes as a slender tubule within the bursal sheath from the blunt anterior end of the uterine cornu to the dorsomedial aspect of the ovary. Prior to puberty, the oviduct has few convolutions and takes a somewhat straight but anterodiagonal course across the ventral surface of the ovary. When the oviduct reaches the medial side it turns abruptly posteriorly to terminate in the dorsomedial aspect of the bursa (Fig. 7). According to Ellenberger (34), the dog is the only species in which the oviduct encircles the bursa. A fibrous band from the anterior end of the uterus, the ovarian ligament, passes over the dorsal aspect of the ovary to attach immediately behind the kidney; medially it bounds a narrow bursal slit approximately 1 mm. in length (Fig. 7). Ellenberger (34) describes the fimbriated end of the oviduct (ostium abdominale tubae) flattening out along the lower border of this slit. The bursal slit changes in size during the estrous cycle. The significance of the bursal slit is not known, but conception after ovarian transplant will not occur if the ovarian bursa is closed completely by sutures (111).

Removal of the ovarian bursa exposes the smooth and glistening ventral surface of the ovary, which resembles a lima bean in shape and size ($1.7 \times 0.9 \times 0.4$ cm. in the beagle). In gross sagittal section, the cortical and medullary zones of the ovary may be distinguished. Histologically, the cortex is covered by a single layer of cuboidal cells showing numerous mitotic figures; budlike cords dip into the underlying collagenous connective tissue, the tunica albuginea. The ovary is devoid of elastic tissue (103). The parenchyma of the cortex is composed of follicular and anovular epithelial cords which arise from the germinal epithelium (15). During their tortuous course into the ovarian stroma these cords lose their continuity with the surface epithelium (38). Follicular cords (Pflüger's tubes) remain in the cortex while epithelial (anovular) cords penetrate deeper and presumably contribute to stromal elements of the medulla (72).

Follicular cords give rise to varying size follicles containing one or more oöcytes (Fig. 4). Numerous oöcytes and primordial follicles abound either singly or in clusters (egg nest) in the outer rim of the cortex. Primary follicles have but a single layer of cuboidal-type granulosa cells surrounding the ovum, while secondary follicles have two or more granulosa layers. Secondary follicles largely increase in size by proliferation of granulosa cells and antrum formation. Frequently, secondary follicles are observed with 2 or more ova; polyovular follicles in

The dog have been reported to contain as many as 11 ova (50). The usual number is 3 to 5 ova (11, 38, 50). For further details concerning the anatomy of the follicle, the reader is referred to Trautmann and Fiebiger (103).

The number of follicles present at birth is drastically reduced at puberty. According to Schotterer (92), who counted the follicles in the ovaries of 5 dogs of different breeds, 700,000 follicles were present at birth and only 350,000 at puberty. Of these, some 1200 to 1300 were vesicular (Graafian) follicles. It has long been known that most of the follicles undergo atresia, yet the process regulating atresia in the ovary has not been explained. As stated by Witschi (112), "atresia degeneration must be regarded as a normal and well-regulated process." The relative number of follicles present in the ovary continues to decrease with advancing age; Schotterer (92) found only 500 follicles in a 10-year-old dog.

The oviduct begins the tubular portion of the genital tract. It is adherent to the ovarian bursa and varies between 5 and 8 cm. in length (94). In cross section, the serosa and subserosa form a distinct connective tissue ring around the tunica muscularis. The tunica muscularis is richly supplied with elastic tissue. Circularly arranged smooth muscle fibers extend obliquely along the tube as well as radiating into the mucosa (103). The lumen of the oviduct is lined by longitudinal folds of substantia propria covered by low columnar epithelium; a submucosa is absent. Although glandular structures are also absent, the mucosal epithelium undergoes striking alterations during the estrous cycle.

The horns of the uterus (Fig. 6) are long and narrow, uniting posteriorly to form the short body. The uterus in cross section, is elliptical in shape and the flattened margins contain longitudinal fibrous bands, the round ligaments (23). Numerous blood vessels traverse the round ligament via the broad ligament to become anastomotic twigs in the subserosa (Fig. 6). Immediately below the subserosa, a relatively thin longitudinal layer of smooth muscle is separated from the thick internal circular layer by the stratum vasculare (103).

The endometrium or mucosa is pale rose in color and presents 4 or 5 longitudinal folds (Fig. 8). Low columnar epithelium invaginates to form uterine glands (42) which number 5 to 10 for each longitudinal fold (11). These glands penetrate in a nearly straight course, extending almost to the muscularis where their terminal ends coil. At certain times, shorter glands appear; these glands are absent in other domestic animals and man (103). Each gland has a lamellar fibroelastic sheath surrounded by tissue spaces and the lamina propria (103).

Grossly, the cervix appears as an oval-shaped mass separating the uterus and vagina. The continuation of myometrial fibers together with fibroelastic tissue forms the body of the cervix, which, in longitudinal section, can be seen protruding into the vaginal lumen. The cervix and the small vaginal folds which surround the external os can be seen upon removing the ventral wall of the vagina (Fig 6). From the external os, the narrow lumen of the cervical canal curves dorsally and anteriorly to open into the uterus at the internal os. According to Arenas and Sammartino (11), the cervical canal does not have a clearly defined tunica propria, merging uterine and vaginal epithelia directly cover the muscularis. They describe the transition between uterine and vaginal epithelium as irregular, that is, folds of vaginal epithelium alternate with endometrial epithelium containing glands which give a positive mucin reaction.

The vagina extends from the cervix to the constricted and poorly defined hymen of the vestibule. A serosal layer exists only in the anterior part, since posteriorly an adventitia occurs as the vagina passes through the pelvic canal. The tunica muscularis of the vagina is similar in arrangement to that found in the uterus, with the exception of an inner longitudinal layer which together with circular muscle fibers forms a loop around the external os (103). The vaginal mucosa is composed of lamina propria and cuboidal type squamous epithelium exhibiting conspicuous longitudinal folds. Lymphocytic follicles and nodules occur in the lamina propria, and two different types of epithelial cells line the lumen. A basal layer of compressed cuboidal cells with scant cytoplasm and dark-staining nuclei is distinguishable from the pseudostratified, acidophilic cytoplasm and pale staining nuclei of the superficial layer (75).

The vestibule is bounded anteriorly by a narrow circular fold of mucosa (hymen) and posteriorly by the vulva (Fig 6, A and C). The outer wall has a sphincter of striated muscle fibers, immediately below are located two distinct bulbs of erectile tissue (Fig 6, D). These bulbs (bulbus vestibuli) unite dorsally and connect ventrally with the erectile body of the clitoris (23). The mucosa of the vestibule contains lymphocytic follicles. The slightly elevated longitudinal folds are lined by stratified squamous epithelium which become cornified at the mucocutaneous junction of the labia.

2 The Estrous Cycle

Grossly, swelling of the vulva and a sanguineous flow herald the onset of proestrus. The vestibule and vagina increase in width (Table

It, and the entire genital tract feels firm and turgid to the touch. Congested blood vessels in the broad ligaments terminate into readily visible uterine vessels as the cornu take on a ventral curvature within the abdominal cavity. Anteriorly, lobules of adipose tissue richly supplied with blood vessels partially conceal the oviducts and ovarian bursa. The entire genital tract is preparing for procreation.

TABLE II
GROSS CHANGES IN GENITAL TRACT AT PUBERTY^a

Organ	Anestrus		Proestrus		Estrus ^b		Metestrus	
	Length	Width	Length	Width	Length	Width	Length	Width
Ovary	1.6	0.9	1.7	1.0	1.7	1.0	1.7	1.0
Oviduct	5.2	0.1	5.8	0.2	0.0	0.3	6.0	0.2
Uterine horn	7.2	0.4	9.0	0.9	9.5	0.9	5.0	1.0
Uterine body	1.5	0.4	1.8	0.9	3.0	1.0	1.9	0.9
Cervix	1.2	0.7	1.2	1.1	2.0	1.3	1.0	0.8
Vagina	5.8	0.6	8.5	2.4	10.0	2.4	0.0	0.9
Vestibule	2.1	0.1	2.0	3.0	2.9	3.2	3.0	3.5

^a Mid-outside measurements in centimeters. Purebred beagles between 7.3-7.8 kg. in body weight, 31-33 cm. in height, and 39-42 cm. in length.

^b Eighteen hours after first acceptance.

a. *Ovary.* Only 7.5% of the average life span of the dog has been spent by the time of puberty (46). Successive estrous cycles cause changes in the genital tract similar to those described at puberty until signs of aging become manifest. This discussion will not deal fully with the complexity of aging phenomena, but rather sketch the genital changes which commonly occur with maturity in the bitch. From puberty to senescence, most organ systems, except the thymus,¹ either retain or gain weight. The ligamentous attachments of the genital tract accumulate large quantities of adipose tissue, especially noticeable about the ovaries. Of more importance are histological changes in the ovary, since this organ is of prime concern in both fecundity and in functional activity of the genital tract. Usually, fertility does not decrease until the dog is 5 years old (82).

Ovarian follicles increase in size so characteristically during proestrus that Arenas and Sammartino (11) identify this phase of the estrous cycle as the "period of follicular maturation." Since most small follicles undergo atresia only a limited number exceed 2 mm. in size. Those attaining 3 to 4 mm. have some 8 to 10 rows of granulosa cells; the theca

¹ At puberty, beagles with a body weight between 7.5 and 7.9 kg. show the following decrease in weight of the thymus: anestrus, 13.8 g.; proestrus, 10.5 g.; estrus, 6.0 g.; metestrus, 4.8 g.

interna increases in vascularity and connective tissue elements. Follicles 4 to 6 mm in size bulge slightly above the surface of the ovary. A unique feature of mature follicles in the dog is a pronounced folding of the granulosa (11, 37, 75) which contain vascular cones of invaginated theca interna (75). In early estrus many follicles have released their ova and show evidence of corpora lutea formation (Fig 6).

It is generally conceded that the dog was the first species in which ovulation was directly observed. Wilhelm Bischoff's classic (19), published in 1845, contains a historical review of the subject and vividly illustrates ovulation and early embryonic development in the dog.

That new oocytes arise from the germinal epithelium during maturity has been reported by Evans and Svezzy (38) and Barton (15). These authors concluded that continuous oogenesis occurs in the dog. During metestrus, germinal epithelium activity increases and is represented by palisading invaginations into the outermost rim of the cortex, in the advent of pregnancy, this activity increases. Barton (15) describes two or three waves of proliferation from the germinal epithelium during each cycle replenishing primary follicles and epithelial elements of the ovary, this occurs largely during late metestrus and anestrus. The concept of continuous oogenesis in the adult has been questioned by other investigators (24). It is proposed by Tsukaguchi and Okamoto (104) as well as Barton (15) that epithelial and anovular cords contribute to interstitial cells in the medulla.

During follicular maturation, the entire genital tract is preparing for reception of ova, an association sometimes referred to as the utero-ovarian cycle (38). However, estrus is not dependent upon the presence of follicles, as demonstrated by Marshall and Wood (70), but, according to Zuckerman (115), is related to the interstitial cells in the ovary and in particular the theca interna. As the follicles mature, the cumulus and contained ovum rotate toward the surface of the ovary (11). At this stage, the walls show elaborate folding of the granulosa and early luteinization (11, 37).

At the time of first acceptance, some follicles contain free-floating ova with corona radiata while others are still connected by the discus proligerus, the first meiotic polar body has yet to be formed (37). Ova are released through a punctate or "pin prick" opening without collapse of the follicle (11). Released ova are $77 \times 90 \mu$, or, including the zona pellucida, $95 \times 110 \mu$ (13), they contain highly refractile globules and are laden with fat (22).

Overwhelming evidence supports the concept that ovulation in the dog occurs spontaneously over the first 3 or 4 days of true estrus (11,

57, 15-69), despite the observations of Whitney (109) that ovulation occurs on the 4th and 5th days of the copulating period. Rowlands (91) found the highest fertility in those dogs bred twice during the first 4 days of estrus. Also, his findings clearly revealed a decrease in conception rate in dogs exhibiting an extended estrous period; for instance, 100% conception was found in eyes lasting 5 to 10 days while only 50% conception occurred when estrus continued 17 to 19 days. Newberry and Gier (78) found evidence of delayed ovulation in females which accepted the male for a period longer than 9 days.

That ovulation is spontaneous in the dog can hardly be refuted, but there is some question as to whether all follicles ovulate simultaneously. The genital tract shown in Fig. 6 was removed 18 hours after first acceptance. At this time, grossly visible corpora lutea (Fig. 6, O) as well as recently ruptured follicles were observed. This observation suggests that ovulation in the dog may be a continuous rather than a simultaneous process. Follicle rupture is believed by Griffiths and Amoroso (45) to extend over a period of 12 to 72 hours; however, some investigators have reported that it occurs simultaneously in all follicles (11, 37). In aged dogs, ovulation may be inhibited, leading to such conditions as ovarian cysts and hyperestrogenism (36).

Ova from recently ruptured follicles are viable for not less than 24 hours and may continue viable into the 4th day (37, 45). Pronuclear division occurs in the middle one-third of the oviduct, segmentation begins in the uterine one-third, and between 5 to 10 days after ovulation the 16-32-cell morula enters the anterior cornu of the uterus (9). The passage of ova through the convolutions of the oviduct is facilitated by muscular contractions, movement of cilia, and possibly an erector action (9). If fertilization does not occur, metestrus or pseudopregnancy ensues.

Corpora lutea form as irregular columns of luteinized cells which fill the follicular cavity by early metestrus (37). In the nonpregnant dog, corpora lutea begin regression at about 20 days of metestrus (37). In the pregnant dog, morphology of the corpora lutea is largely maintained until the end of gestation (75). Corpora lutea spuria are evident on succeeding estrous periods.

With advancing age, changes occurring in the ovary and uterus (Fig. 11) during the estrous cycle become less pronounced. Grossly, the ovary in the aged dog has a cauliflowerlike surface with deep crevices separating varying-sized nodules (Fig. 8). Such ovaries contain atretic follicles, dense connective tissue, scarred remnants of previous corpora, and a reduction in medullary stromal elements (Fig. 9). As shown in

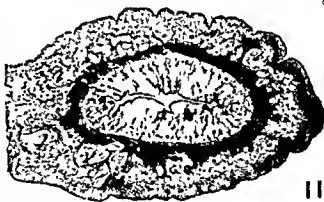
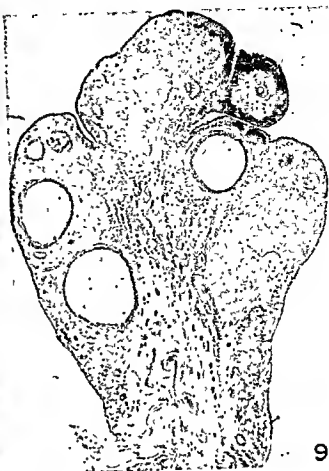
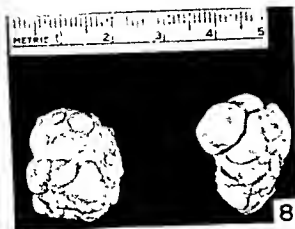


FIG. 8. Ovaries from 12½-year-old beagle which was in continuous anestrus for a period of 2 years. Note cauliflowerlike surface.

FIG. 9. Cross section of ovary from 8-year-old beagle dam. Note irregular surface and variation of parenchymal elements in the cortex and relative absence of stromal elements in medulla. Bouin's, paraffin, hematoxylin and eosin. Magnification: $\times 9$.

FIG. 10. Surface of ovary shown in Fig. 2. Note pseudostratified columnar epithelium with cilia-like protrusions on surface; section 8 microns thick. Magnification: $\times 850$.

FIG. 11. Cross section of uterus from 6-year-old dam in anestrus. Note thick-walled arterioles in stratum vasculare. Formol, nitro-cellulose, hematoxylin and eosin. Magnification: $\times 9$.

the germinal epithelium becomes pseudocolumnar in type with the protrusions, and shows few mitotic figures. Morphological changes in the ovary reflect irregularities in the estrous cycle and the reduction in reproductive fitness as the dam advances in age (2). These irregularities occur in both virgin and parous dogs (69). Whitney (111) demonstrated that aged ovaries transplanted into a young recipient could resume normal reproductive function. It is well known that a distinctive climacteric does not occur in the dog, but rather estrous intervals gradually lengthen. Yet, when estrus does occur in the aged dog, fertility may not be seriously affected (2). Mulligan (75) cites one exceptional case in which the estrous cycle continued regularly until the dog was 20 years of age.

Little attention has been given to the rete ovarii, but it has been suggested by Arenas and Sammartino (11) that this structure may be functional. These authors could not distinguish cyclic activity in the rete; however, during anestrus the lumen enlarged and sometimes it assumed a labyrinthian (laberintia) structure.

b. Oviduct. The oviduct becomes convoluted and thickened at proestrus; this heightens at estrus (Fig. 6, U). Arenas and Sammartino (11) describe an increased folding of the mucosa which shows spike-shaped (intercalated) and columnar cells during proestrus, with increasing numbers of secretory cells as estrus intervenes. Material studied in our laboratory shows rather short ciliated epithelium in the fimbria and anterior oviduct which become less numerous toward the uterine extremity. After puberty, convolutions are a permanent feature of the oviduct, but the thickness shows variations during the estrous cycle.

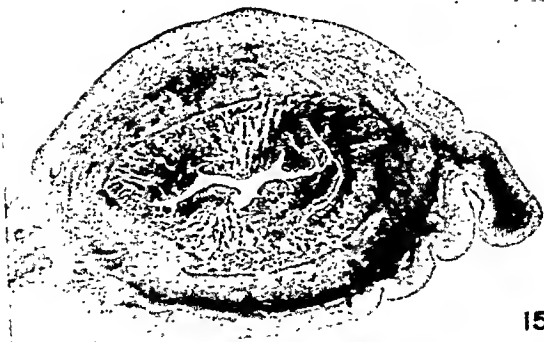
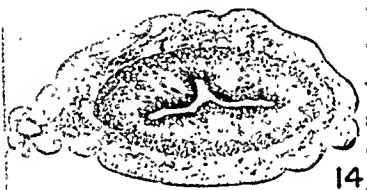
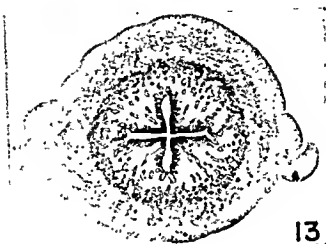
At proestrus, the bursal slit increases in length from 1.0 to 3.0 mm. in the beagle, and a reddish-colored mass (rôtiche Masse) protrudes into the peritoneal cavity (Fig. 7). In section, this mass constitutes an enlarged portion of the fimbria (fimbriata mucosa). The fimbria re-

FIG. 12. Cross section of uterus. Anestrus. Bouin's, nitrocellulose, hematoxylin and eosin; section 12 microns thick. Magnification: $\times 10$.

FIG. 13. Cross section of uterus. Proestrus. Note increase in size and tubular appearance resulting largely from congestion and edema. Bouin's, nitrocellulose, hematoxylin and eosin; section 12 microns thick. Magnification: $\times 10$.

FIG. 14. Cross section of uterus. Estrus. Note further enlargement due principally to hypertrophy of both myo- and endometrium. Bouin's, nitrocellulose, hematoxylin and eosin; section 12 microns thick. Magnification: $\times 10$.

FIG. 15. Cross section of uterus. Metestrus. The striking increase in diameter is accompanied by a marked decrease in length of the cornu. Bouin's, nitrocellulose, hematoxylin and eosin; section 12 microns thick. Magnification: $\times 10$.



...mag metestrus, enabling the ovarian surface to be viewed through the bursal slit; this is especially noticeable during pregnancy. The slit partially closes at anestrus and fluids injected into the bursa may be retained for several hours.

c. *Uterus*. Gerlinger's thesis (42), and subsequent reports (11, 37, 72, 75) have greatly clarified changes occurring in the uterus and vagina throughout the estrous cycle. This discussion will summarize changes manifested in the uterus (Figs. 12, 13, 14, and 15), cervix, vagina, and vestibule. At the onset of proestrus, engorged uterine vessels are especially noticeable in the stratum vasculare as thick, muscular-walled arterioles. Hypertrophy of endometrial components is characterized by congested capillaries, interstitial edema, and mitotic figures in the low columnar epithelium lining the lumen. The mucosa shows budlike projections of epithelium penetrating into the lamina propria. Often, crescent-shaped nuclei surround the more superficial aspects of these buds which are clearly seen in histological sections. Congested capillaries with extravasated erythrocytes are evident immediately below the epithelium. The amount of congestion increases deeper into the subepithelial connective tissue, forming a hyperemic zone surrounding the lumen (37). Extravasated erythrocytes from the hyperemic zone apparently migrate through the lining epithelium into the uterine cavity by diapedesis, since focal bleeding is not a common observation in this species (11, 37, 72). During estrus, erythrocytes within the lumen of the uterus have become greatly reduced in number, and the endometrium shows extensive glandular growth toward the end of estrus and early metestrus. The complex glandular structure of the endometrium is about 1.0 mm. in thickness and consists of three layers: (a) superficial sacs, "crypts of Biscoff," or the short glands; (b) middle layer of gland luminae and stromal elements; and (c) deep layer of coiled tubular glands (11).

About the 20th day of metestrus, uterine glands begin to atrophy, the lumen enlarges, and congestion wanes (37). As shown in Table II, the uterine cornu are shortened in length to about one-half that found at estrus, but the diameter is maintained. Endometrial regression requires 80 to 90 days to return to the state characteristic of anestrus (37). If pregnancy ensues, the endometrium undergoes further hypertrophy (11, 33, 75); however, endometrial regression is completed at about the same time as in the nonfertile cycle (37). Pigmentation-identifying sites of fetal implantation persist (33).

The cervix increases in size at proestrus principally by congestion and interstitial edema. The mucosal lining is thrown into numerous

folds and the previously almost closed lumen becomes dilated and filled with eosinophilic-staining material. The cervical canal increases in size and fluids can pass readily into the uterus during estrus and metestrus. At the close of metestrus, the cervical canal is virtually sealed and only occasionally can microscopic debris be noted within its lumen.

d. Vagina. At proestrus, the lumen of the vagina and the external os of the cervix show bulging folds of mucosa. The surface of such folds appears "puffed"; some reach 1.0 cm. or more in width. The vaginal wall shows congestion and interstitial edema. Epithelium, lining the external os of the cervix and the vagina, undergoes pronounced hyperplasia. The flattened 5- to 8-cell layer of stratified squamous epithelium seen in anestrus now becomes highly stratified with papilla projecting into the lamina propria. By mid-proestrus, when the sanguineous flow gains momentum, the vaginal folds lose their "puffy" appearance. The most distinctive feature of the vaginal mucosa during estrus is extensive wrinkling (*rugae*) which becomes less pronounced toward the cervix (Fig. 6). Evans and Cole (37) describe the epithelium lining the vagina as 12 to 20 cells in height, producing a stratum corneum which desquamates until the latter half of estrus. The height of epithelial activity during the first part of estrus is followed by both a reduction in the thickness of the vaginal wall and the squamous epithelium. Concomitantly, congestion and edema recede so that by metestrus, the vaginal folds have lost their wrinkled appearance. During late metestrus and anestrus, the vaginal mucosa shows small, smooth longitudinal folds.

e. Vestibule. The vestibule does not show mucosal wrinkling during proestrus and estrus. However, during these phases the congested and edematous wall reveal hyperplasia of the lining epithelium, but not to the extent found within the vagina. The stratum corneum in the vestibule is less developed, with correspondingly few desquamated cells. Arenas and Sammartino (11) believe that the epithelium of the vestibule does not serve as a means for recognizing phases of the estrous cycle. Mucosa in the vestibule and vagina increases in sensitivity during proestrus and estrus. Hypertrophy of lymphocytic follicles and nodules may be seen grossly within the vestibule as miliary stippling.

3. Vaginal Smear

Evans and Cole (37), using a large number of dogs, found characteristic elements in vaginal smears which coincided both with the external signs of estrus and morphological changes of the genital tract throughout the estrous cycle. These observations have been confirmed by other investigators (45, 47). However, Papanicolaou and Blau (79)

or normal virgin beagles maintained in our kennel have not clinical signs resembling pregnancy. Therefore, the authors feel that the term metestrus should be reserved for the luteal phase of the estrous cycle when clinical symptoms resembling pregnancy are not evident. On the other hand, the terms pseudo- or phantom pregnancy should be used to denote symptoms of pregnancy in the absence of conception; and pseudocyesis, its exaggerated form.

The diversity of symptoms exhibited in pseudopregnancy and pseudocyesis is explained by the phenomena of spontaneous corpora lutea formation and extended function (68). During the 5th week following estrus, pseudopregnancy may be recognized by an increased width of the uterine cornu, which may be detected upon abdominal palpation (47). Visible symptoms of pseudopregnancy, whether mild or severe, include the gradual deposition of abdominal fat, and mammary development followed by nervousness, panting, and nesting between the 60th and 70th day (87). In severe cases (pseudocyesis), the dog may show "motherly instincts" such as, refusal of food, protection of inanimate objects, or the adoption of foster young. If allowed to suckle, lactation may continue for several weeks (60). The formation of colostrum has not been observed in cases of pseudocyesis brought to the attention of the senior author. In chronic cases, the only recommended treatment for this condition is spaying (60).

During metestrus most dogs show a chocolate-colored vaginal drainage and a flabby, enlarged vulva. Most, but not all dogs, exhibit "slight" but palpable mammary development for 6 to 10 weeks following estrus, while in pseudopregnancy full lactation may occur.

In bred females, corpora lutea retain functional activity until mid-gestation, followed by slow regression (24, 69). Pregnancy may be diagnosed by palpation on the 30th day, but not with any degree of accuracy in all cases until the 40th day (61). Neither vaginal smears (37, 47, 63) nor urine pregnancy tests, as applied to women (53), are reliable indices for diagnosing pregnancy in the dog. The dam does not show behavior characteristic of pregnancy until late gestation (10). Therefore, there is a diagnostic problem in differentiating between pseudopregnancy and pregnancy. Whitney (108) cited several cases in which pregnancy was terminated by resorption of fetuses and symptoms of pseudopregnancy intervened.

The following discussion concerns the anatomical changes, related to corpus luteum function and regression, which occur in the genital tract of the bitch during the luteal phase. Arenas and Sammartino (11) describe corpus luteum formation beginning shortly before ovulation. After

the spontaneous release of ova, secondary folds of granulosa with papillary branching penetrate into and fill the follicle cavity. During this process, the granulosa shows vacuolization and accumulation of lipid droplets; lipids are first seen in the peripherally located cells. These luteinized cells are round or polygonal in shape, containing fine cytoplasmic droplets rich in lipids. Theca luteal cells may appear as islets surrounding the corpora. According to Arenas and Sammartino (11), in the dog, the theca interna does not contribute to corpus luteum formation to the extent found in other species, e.g., man.

The mature corpus luteum is 5 to 6 mm. in diameter, having the gross appearance of a compact reddish-yellow nodule. Histologically, it appears as a solid or nearly solid mass of luteal cells separated into ill-defined lobules by collagenous connective tissue and capillaries; it is surrounded by loose stromal elements and congested blood vessels. According to Mulligan (75), corpus luteum formation is completed by the 13th day of metestrus. Atrophy or regression occurs slowly, being characterized by fatty degeneration, nuclear changes, and a decrease in cellular volume (11). According to Evans and Cole (37), regression of corpora lutea was recognized on the 20th day, and the genital tract slowly began involution to the quiescent state characteristic of anestrus; this process required 80 to 90 days from the time of last acceptance. Actually, morphological evidence of regression first becomes discernible in the endometrium before it can be recognized in the corpora lutea (11). Luteal islets can often be found in the ovary on succeeding estrous periods.

In clinical pseudopregnancy, visible mammary development may vary from slight to that of full lactation. The average dog has 8 to 10 mammary glands located in double rows, often asymmetrically, along the ventral thorax and abdomen (23). Until puberty, each gland consists of rudimentary ducts of epithelial cords with or without lumina (103). At puberty, this simple branched tubular gland grows into a compound alveolar gland. By 30 days, growth of the mammary tree is complete (105). Turner and Gomez (105) have compared mammary development in both nonbred and bred dogs; in both instances, alveolar secretion was beginning by the 40th day following estrus. In the nonpregnant dog, alveolar secretion did not occur to the extent found in the pregnant dog; however, intense lactation could be induced in the virgin dog by injections of prolactin. In a report on pseudopregnancy in the dog, Heape (52) also mentioned the phenomena of milk secretion in the mule and virgin donkey, and noted that the mammary glands in women began developing prior to the menstrual period.

...t parenchymal elements of the mammary gland occurs in the same fashion after the fertile and nonfertile cycle. Bred females require a few days longer than nonbred, the difference in time required being the degree of functional activity reached. In metestrus, mammary secretion is represented by a slight amount of serous fluid. In pseudopregnancy, secretion consists of milklike products. This is accentuated in pseudocyesis when nursing is possible. In pregnancy, mammary development and colostrum formation are at their height.

IV. FACTORS INFLUENCING THE ESTRUS CYCLE

A. Environment

Although environmental factors apparently have little or no effect on the estrus cycle of the dog, it has long been known that the incidence of estrus increases at certain times of the year. We shall, therefore, include in this discussion some exogenous forces which may influence the rhythm of the estrus cycle.

1. Season

The wild dog (*Canis azaroe*) mates only in winter (69). Domestic dogs may breed at any time of the year, but the frequency of estrus has a bimodal pattern (13), i.e., most dogs come into estrus during early winter and late spring (47, 69, 82, 100). Heape (51) observed that winter estrus in certain breeds was of shorter duration than summer estrus. Engle (35), on examining breeding records of a large number of Pekingese, Cocker Spaniels, Setters, and Great Danes, found no significant seasonal or geographical variation, but, as pointed out by Laing (61), this could have been influenced by breeders attempting to meet the most popular times for sales. Our kennel records from some 400 female beagles confirm the year-round occurrence of estrus, with greater frequencies during early winter and late spring. Apparently the amount of light received is not a factor, since solar energy is lowest in winter and relatively high in spring, yet the frequency of estrus is high at both seasons.

2. Nutrition

The effect of diet on the estrus cycle in the dog has been given little attention. Laing (61) states that reproduction is affected by both inanition and adiposity; however, it is difficult to say with certainty whether excessive body fat is a result or a cause of reproductive failure. That quality of the diet is equally important for normal reproductive performance is discussed in Chapters 3 and 22. Contaminated or

adulterated feed may possibly affect the estrous cycle, as has been shown by progressive and irreversible infertility in a colony of guinea pigs fed on an estrogen contaminated ration (113)

3 Ecology

From time to time, breeders have expressed the view that one dog in season causes others to come into heat. The authors have not found any information to corroborate this concept, on the contrary, our experience suggests that this is not the case. Over a 5-year period, observations of some 400 female beagles kept in large, outside runs as paired mates in no way indicated that direct contact influenced the occurrence of estrus. In our observation, the onset of normal estrus can only be explained on an individual basis, i.e., the inherent regulatory mechanism controls the monestrous cycle in dogs rather than social factors.

4 X-Irradiation

Whole-body X-irradiation (250 kvp, 30 ma) of 100 r and 300 r administered to beagles at puberty did not significantly alter the estrous interval (3, 8). Since X- and gamma rays are similar in biological effect ($RBE = 1$), it may be concluded that female dogs surviving lethal and sublethal whole-body exposure by either ray may not be seriously affected as concerns the periodicity of estrus.

B Physiology

During the first three decades of the century, when the role of the ovary as an organ of internal secretion became established, the analogy between proestrous bleeding in the dog and menstruation in primates was of great interest. In 1906, Marshall and Jolly (69), as a result of experiments in the bitch, concluded "The ovary is an organ providing an internal secretion which is elaborated by the follicular epithelial cells or by the interstitial cells of the stroma. This secretion circulating in the blood induces menstruation and heat. After ovulation, which takes place during estrus, the corpus luteum is formed, and this organ provides further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy." Although this concept of ovarian function proved to be basically correct, proestrous bleeding and menstruation were subsequently shown to be unrelated phenomena (38, 72, 88). Since this time, the dog has been largely neglected as an experimental animal for the study of the physiology of reproduction. The voluminous literature responsible for present-day knowledge of the endocrine control

tion is comprised chiefly of experimental work in laboratory animals with a short estrous cycle and domestic animals of economic importance. Comparatively little quantitative data are available on the dog. The following section reviews reported effects of extirpation of the endocrine glands, replacement therapy, and hormone injections on the reproductive system in the bitch.

1. *Hypophysectomy, Ovariectomy, Adrenalectomy*

The results of hypophysectomy on the genital system of the bitch have been reported by a number of investigators (12, 17, 18, 26, 32). These reports were reviewed by Arenas and Sammartino, and their own observations on postoperative changes in the mature animal were described in some detail. Hypophysectomy of immature female dogs results in cessation of growth and failure of sexual maturity. In such animals, the ovaries are small and hypoplastic; the uterus remains infantile, and secondary sex characters do not appear. After hypophysectomy of mature females, the degree of atrophy observed varies according to the time elapsed and the phase of the cycle when the operation was performed. Follicles attain a maximum size of 0.5 mm. Regressing corpora lutea may persist, in some cases, until the 40th day, and the anovular medullary cords have almost completely atrophied by the 75th day. Uterine involution resembles the physiological process, but compared with the anestrus uterus, a marked reduction in size becomes evident by the 80th day. Hypophysectomy of the pregnant bitch at any stage of gestation results in abortion (12, 17, 18, 54).

Reports concerning section of the pituitary stalk in the dog have varied from cessation of sexual function to that of no effect. However, it has been demonstrated in other animals that if the vascular connection between the hypothalamus and the anterior pituitary is left intact, or regeneration occurs, pituitary function is not impaired. This may explain the conflicting results following transection of the pituitary stalk in the dog (48).

According to Arenas and Sammartino (11), ovariectomy in the immature bitch results in no appreciable regressive changes in the uterus. Mature females, spayed during anestrus, show a thinning and flattening of the uterus. Ovariectomy during estrus is characterized by an intense shrinking of the endometrium and myometrium and during pseudopregnancy by an abrupt collapse of the endometrial folds. These changes are completed by 28 days. The pregnant bitch may be ovariectomized during the latter half of gestation without causing abortion (71, 87).

Bitches adrenalectomized during "heat" (83, 90) or during pregnancy (89) survive longer than those adrenalectomized during anestrus, presumably due to the salt- and water-retaining effects of the sex steroids (101, 102). The effective agent for survival is probably progesterone secreted during metestrus or gestation (80).

2. Replacement Therapy

a. Gonadotropins. Several anterior-pituitary gonadotropic preparations have been used for replacement therapy after hypophysectomy. However, hormonal requirements for a specific gonadotropic effect; i.e., follicular maturation, estrogen production, ovulation, or corpora lutea formation and function have not been established. Daily injections of rabbit pituitary gland in a hypophysectomized puppy caused vulva swelling in 48 hours and acceptance of the male after 18 days; estrus persisted throughout 4 months of injection (85). Mature hypophysectomized females injected daily with an alkaline extract of bovine pituitary gland showed large numbers of follicles about 1 mm. in size and a uterine response equivalent to full proestrus (11). "Genital turgescence" but not heat was obtained in mature hypophysectomized bitches with commercial sheep and beef anterior pituitary gland extracts (44). Presumably, the doses employed were too small to provoke more than a minimal ovarian response. Chorionic gonadotropin has been shown to be ineffective in stimulating the genital system of hypophysectomized puppies. However, a combination of chorionic gonadotropin and a hypophyseal extract low in gonadotropic potency acted synergistically to activate the ovary (37a). In a dog with a well-developed mammary tree, copious lactation was induced with a crude prolactin preparation one week posthypophysectomy (67).

b. Ovarian Hormones. Ovaries from normal dogs in estrus, grafted into the groin, peritoneal cavity, or abdominal muscles, induce estrus in spayed recipients (69). Proestrous bleeding and mating can be obtained in castrate females with estrogenic hormones (58, 62, 72, 88). Kunde *et al.* (58) injected female dogs, 18 days following spaying, with estrogen prepared from human pregnancy urine. Doses of 100 R.U. (rat units) (Allen-Doisy) daily for 3 days and 200 R.U. daily thereafter, produced vulva swelling on the 4th day, bleeding on the 6th day, and acceptance of the male on the 7th day. Similar results are obtained in hypophysectomized dogs (88). The minimal effective dose for proestrus changes in the uterus was found to be 200 I.U. of estrin per day (11).

Areus and Sammartin (11) studied the action of progesterone on the estrogen-primed uterus in castrate bitches. They concluded that a

combination of estrogen with progesterone would be necessary to simulate changes occurring during the luteal phase of the cycle, but were unable to arrive at the optimum combination to produce this effect. Presumably progesterone is also necessary to obtain mammary growth, since glandular proliferation does not occur in castrate bitches after administration of estrogen (105).

Whitney (110, 111) reported that when both recipient and donor were in proestrus or estrus at the time of operation, ovaries from one breed of dog were successfully grafted into the ovarian bursa of another, and that normal estrous cycles resulted in the recipient. Cyclic behavior characteristic of the donor was resumed. Ovaries transplanted from an aged bitch into a young recipient showed a reversal of aging phenomena. Successful breeding was reported in one case in which the proper anatomical relationships were maintained, specifically, a patent bursal slit (Section III, C).

3. Hormone Injection in the Intact Animal

a. *Pituitary Gonadotropins.* Activation of the ovary of the intact bitch during anestrus with several types of gonadotropins of pituitary origin has been reported. Ovarian response to whole anterior pituitary gland extracts varies according to potency in the separate gonadotropic fractions, dose, and length of injection. Results have varied from proestrus bleeding (44, 72), follicle growth, and proestrus development of the uterus and vagina (11), to the induction of heat, ovulation, and copulation resulting in pregnancy (66).

Estrous cycles of normal duration and intensity can be induced in the anestrus bitch with extracts of menopausal urine (62, 99). Leathem (62) reported that the injection of 75 R.U. per day of "gamone" into mature anestrus dogs until proestrus bleeding or mating was induced caused extensive follicular stimulation, followed by corpora lutea formation and uterine changes typical of metestrus; ovulation did not occur. The additional injection of 500 R.U. of chorionic gonadotropin ("follutein") on the 1st day of estrus resulted in ovulation in 1 out of 3 cases. Proestrus bleeding and mating were induced in an immature dog, but comparatively few mature follicles were observed in the ovaries. Comparable results in mature animals were obtained with extracts of normal human male urine (63). Copious lactation can be induced in the bitch during metestrus with prolactin preparations (67, 105).

b. *Placental Gonadotropins.* Equine gonadotropin (PMS) and chorionic gonadotropin (HCG), are widely used therapeutically in

veterinary medicine for the treatment of ovarian dysfunction (87) PMS is used principally for follicle-stimulating activity, but can induce a complete estrous cycle, including ovulation, in most species HCG is generally used to induce ovulation and as a luteinizing hormone In some species, however, it acts synergistically with endogenous gonadotropins to induce an estrous cycle in immature or anestrus animals "Heat" and mating have been reported in anestrus bitches after injection of PMS (44, 47b, 93) For treatment of sterility, HCG is usually added to ensure ovulation (20, 28, 93) Scorgie (93) reported the successful treatment of "sterility due to functional causes" in bitches of a number of breeds by the injection of a single dose of 187 M U (mouse units) PMS ("antostab") and 50 M U HCG ("phytostab") However, since PMS alone is effective for the treatment of sterility in other domestic animals (87), it probably would be equally so in the bitch at the proper dose levels "Heat" has been produced in anestrus bitches by injection of HCG, but a normal estrous cycle did not result (63a) Daily injections were given until acceptance of the male, however, neither ovulation or metestrus followed nor did the vaginal smears or endometrium duplicate proestrous changes during the normal cycle

c *Sex Steroid Hormones* The action of the sex steroid hormones in the intact animal is extremely variable and is largely dependent on the physiological state at the time of injection and the dose level given In addition to their effect on the secretion of the gonadotropic hormones from the pituitary gland, they may act either synergistically or antagonistically with each other to produce a given effect Estrogens, injected at "physiological" or pituitary stimulating levels, can incite an estrous cycle in mature anestrus animals Intramuscular injections of stilbestrol dipropionate in ascending doses from 0.05 to 0.5 mg at intervals of 4-5 days until vulva-swelling or bleeding appears have been found to induce a complete estrous cycle and ovulation in anestrus, parous, and nulliparous bitches Results were much less reliable in maidens (47)

It is well established that the daily injection of estrogens will produce stimulation of the external genitalia, proestrous bleeding, and mating in anestrus and castrate bitches (58, 62) The minimal dose for proestrous changes in the endometrium is apparently 200 I U (11) At these dose levels, inhibition of release of FSH from the pituitary gland occurs The ovary either is not affected, retaining the anestrus appearance (11, 14, 41, 62), or after long periods of injection may show signs of atrophy Kunde *et al* (59) reported a decrease in size of the ovaries and an arrest in development of follicles in immature females

receiving 400 R.U. of estrogen per day for 6 weeks and a decrease in size of the anterior pituitary gland in 1 animal injected for 17 weeks. Estrogen therapy is recommended for the treatment of hyperestrinism in the bitch where hyperactivity of the pituitary gland may be the causative factor (20, 87).

Estrogens injected during the luteal phase of the cycle will cause uterine bleeding and degenerative changes in the endometrium (88). They are used for the treatment of pseudocystitis, daily injection of 0.1-1.0 mg. of diethylstilbesterol being the recommended dose (20). Large doses of estrogens will prevent conception or cause abortion (25, 55, 87).

Lactation has been observed in female dogs injected during anestrus with estrogens at levels causing proestrous uterine changes (14, 59); presumably release of prolactin from the pituitary gland was induced. Inhibition of lactation during pseudopregnancy or after parturition can be obtained with estrogens or with testosterone (20). Possibly the diametrically opposite effects of estrogen on lactations can be explained on the basis of the amount administered. Bratt and Burch (25) reported reduction in size of the mammary gland and suppression of lactation 24-48 hours after injection of 25 mg. of an "implant" type testosterone preparation.

Progesterone in doses of 10 to 50 mg. per day has been recommended to counteract the endometrial and vaginal changes during hyperestrinism. Doses of 5-25 mg. several times a week are recommended for maintenance of the uterus in cases of abortion due to faulty corpora lutea formation or function. Progesterone given during proestrus will delay estrus in bitches for as long as the treatment is continued. Murray and Eden (77) recommend a dose of 1 to 1.5 mg./lb. of body weight of "repositol" progesterone repeated at 2-3-week intervals.

C. Pathology

This section deals with pathological conditions or states related to the estrous cycle of the dog. There is a problem, not in the recognition of a pathological condition, but in determining the narrow threshold between the normal and the abnormal. For detailed accounts, the reader is referred to texts dealing with the pathology of the reproductive system (21, 65, 76, 87, 96).

Estrus without bleeding, false heat, polyestrus, prolonged estrus, and prolonged anestrus are no doubt due to endocrine imbalance. The bitch in estrus without visible signs of bleeding accepts the male, usually unexpectedly, and conception almost invariably follows. Cases of this type brought to the attention of the senior author did not show the

usual enlargement of the vulva, but the female accepted the male without objection. *False heat*, or a "split" estrous cycle, is commonly seen in females approaching puberty and has been observed in older dogs at periodic intervals between true estrous cycles. Dogs in false heat show vulvar swelling and sanguineous discharge without desire to accept the male. False heat usually continues for 3 to 5 days, then subsides until the advent of true estrus. *Polycstrus* is rare in the dog. When it does occur, the short intervals between estrus may persist for years. The bitch will accept the male, but matings are infertile. In our kennel, a female was bred every 3 to 4 weeks over a 2-year period before she finally conceived. She subsequently weaned a litter and resumed a normal estrous rhythm. Females in *prolonged estrus* accept the male for a period of 15 to 30 days. Hancock and Rowlands (47) observed that fertility decreases progressively when the female accepts the male beyond 10 days. *Prolonged anestrus* may continue for years, in our experience, this condition is most frequently seen in obese dogs.

Some of the more common abnormalities in the dog are pseudopregnancy and pseudocyesis, which are probably forerunners of endometritis, pyometra, or mammary tumors. It will be recalled (Section III, D) that most dogs in metestrus do not show visible signs characteristic of pregnancy, also, atrophy of the endometrium could be recognized before corpora lutea regression (11). On the other hand, clinical pseudopregnancy is characterized by endometrial hyperplasia and mammary development (60). Necropsies of pseudopregnant dogs show the uterus filled with a milky fluid (39). Apparently, the accumulation of uterine fluid and the failure of the cervical canal to reduce in size create conditions favorable for uterine infection. Although experimental evidence is lacking, it is generally believed that bacteria from the vagina enter the uterus and cause endometritis. Leibold (64) isolated pyogenic bacteria from the external os, and Demmel and Witzigmann (30) demonstrated that saprophytic bacteria from the vagina were capable of causing uterine infection. Once infection has been established, the condition may become chronic and result in sterility. In cases of low-grade endometritis, estrus—whether spontaneous or induced—may eliminate the infection. Some nonsuppurative conditions of the uterus (endometrial hyperplasia) are characterized by innumerable endometrial cysts containing a clear or pale yellow, watery fluid (21). Pyometra, an accumulation of suppurative products (31, 98), probably results either directly or indirectly from ovarian dysfunction (36, 73).

It is well known that mammary neoplasms are commonly found in virgin dogs. Riser (86) studied a large number of mammary tumors

in nulliparous dogs and, almost without exception, they were observed in dogs which had frequently shown clinical signs of pseudopregnancy. In his study, mammary tumors accounted for 30.8% of all tumors observed. From 266 tumors of the genital tract examined by Cotchin (29), 222, or 83%, were found in the mammary gland. The frequency of mammary tumors increases in dogs above 5 years of age. A correlation also exists between irregularities in the estrous cycle and the incidence of tumors (21).

V. BREEDING, WHelpING, LACTATION, AND PUPPY PRODUCTION

The objective or purpose of estrus is to reproduce the species. In the dog, mating or breeding is somewhat unique, since approximately 20 minutes is required to complete the act. During pelvic thrust, erectile tissue (bulbus glandis) of the penis enlarges within the vagina resulting in a "tie" or "coital-lock." When "coital-lock" has been achieved, the male dismounts and faces away from the female while ejaculation continues. Semen averaging about 9.5 ml. in volume (pH 6.75) is released in three fractions: (1) waterylike fluid; (2) viscid, white fluid containing sperm; and finally, (3) a larger volume (3-30 ml.) of prostatic secretion (49). The third fraction requires the longest time (average, 15 minutes) to be ejaculated; this secretion is slightly basic (pH 7.20) and apparently activates spermatozoa (49). Density of spermatozoa ranges from 62 to 640 thousand per microliter (average = 275,000 μ l.), with 4.8% abnormal sperm and dead sperm not exceeding 20% (47). Sperm can survive 82 ± 12 hours within the female genital tract (45). Griffiths and Amoroso (45) express the opinion that restraint of the female for purpose of copulation seriously reduced fertility by interfering with reflex stimulation of the genital tract.

Fertility in the dog is usually high. Among mongrel dogs a 90% fertility can be expected (40). Rowlands (91), using purebreds, observed 60% conception after 1 mating, 87% after 2 matings and 100% conception following 2 matings at 48-hour intervals during the first 4 days of estrus. In beagles, we found 91% conception when females were bred twice on alternate days from the onset of estrus.

The number of fetuses varies between 1 and 15, or more, depending upon the breed and individual. The number of young per litter is not dependent upon polyovular follicles in this species (50, 57). Fecundity is apparently related to the inherent ability of the individual dog and proper time of breeding. Ectopic pregnancy is extremely rare in the dog, although ovarian, bursal, and oviduct pregnancies have been reported (21). Fetal implantation occurs 18 to 20 days after mating (95).

Pregnancy can be accurately diagnosed by palpation during the 5th week of gestation (47) As the abdomen enlarges during late gestation, the teats become turgid and develop a bluish pigmentation Gestation normally ranges from 58 to 63 days (13, 33) Pearson and Pearson (82) observed the most probable whelping date was 61.41 ± 2.14 days after first mating, these authors could not explain variation in the length of gestation by litter size, number of pregnancies, or by age of the dam Similar observations have been reported by Rowlands (91)

The dam approaching term becomes restless, pants, and seeks a secluded spot for nesting Once definite signs of labor are evident, the female rarely changes the selected site for delivery Labor is characterized by rhythmic uterine and abdominal contractions and the periodic expulsion of a fetus Pups are normally delivered in a definite pattern, i.e., during whelping, the dam moves around the perimeter of her nest, stopping only to free the newborn pup from fetal membranes which she usually ingests Most dams complete whelping 3 to 6 hours from the onset of labor (2, 97, 114)

The delivered pup immediately seeks the udder for nursing The dam's milk contains about twice the total solid content of cow's milk It is especially high in albumin (45%) and fat (90%) (27) Litters are usually weaned when the pups are 5 to 7 weeks of age The successful raising of the pup to weaning age, largely the concern of the dam, has been discussed elsewhere (2) It is during the transitional stages occurring at birth and again at weaning that puppy losses are greatest The 3 year old dam excels in reproductive performance, apparently, at this age she is at her prime Until the litter is successfully weaned, one third of all puppies whelped may be expected to succumb from any one of several causes (2, 91)

ACKNOWLEDGMENTS

The authors wish to thank Drs Miriam E Simpson and Herbert M Evans whose suggestions and constructive criticism are deeply appreciated We are also indebted to Mr William Cee Mrs Gabriele Levine, and Mrs Virginia Gibson for their aid in surveying the literature and in reviewing the manuscript

REFERENCES

- 1 Amoroso E C, in *Marshall's Physiology of Reproduction* (A S Parkes ed), 3rd ed Vol II, p 127 Longmans Green, New York 1952
- 2 Andersen A C, *J Am Vet Med Assoc* 130 151 (1957)
- 3 Andersen A C, in 6th Annual Progress Report, A E C Project No 4, Univ Calif Davis, California 1957
- 4 Andersen A C *J Am Vet Med Assoc* 132 95 (1958)
- 5 Andersen A C, and Cee W *Mich State Univ Vet* 18 18 (1957)

- 6 Andersen, A. C., and Gee, W., *Vet. Med.* **53**, 135 (1958).
7. Andersen, A. C., and Hart, G. H., *J. Am. Vet. Med. Assoc.* **126**, 366 (1955).
- 8 Andersen, A. C., and Shultz, F. T., unpublished, 1958.
9. Andersen, D., *Am. J. Physiol.* **82**, 557 (1927).
- 10 Anderson, O. D., *Am. Anat. Mem. No.* **19**, 647 (1941).
11. Arenas, N., and Sammartino, R., "Estudio Experimental Sobre los Organos Genitales de la Perra." Aniceto Lopez, Buenos Aires, 1938.
12. Aschner, B., *Wein. klin. Wochschr.* **49**, 1730 (1909).
13. Asdell, S. A., "Patterns of Mammalian Reproduction." Comstock, Ithaca, New York, 1946.
14. Asdell, S. A., and Marshall, F. H. A., *Proc. Roy. Soc.* **B101**, 185 (1927).
15. Barton, E. P., *J. Morphol.* **77**, 317 (1945).
16. Beach, F. A., and Cilmore, R., *J. Mammal.* **30**, 391 (1949).
17. Bell, W. B., *Quart. J. Exptl. Physiol.* **11**, 77 (1917).
18. Bell, W. B., "The Pituitary." Baillière, Tindall & Cox, London, 1919.
19. Bischoff, T. L. W., "Entwicklungsgeschichte des Hundes." Friedrich Vieweg und Sohn, Braunschweig, 1845.
20. Bloom, F., in "Canine Medicine" (H. P. Hoskins, J. V. Lacroix, and K. Mayer, eds.), p. 244. American Veterinary Publications, Evanston, Illinois, 1953.
21. Bloom, F., "Pathology of the Dog and Cat: the Genitourinary System, with Clinical Considerations." American Veterinary Publications, Evanston, Illinois, 1954.
22. Boyd, J. D., and Hamilton, W. J., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), 3rd ed., Vol. II, p. 1. Longmans, Green, New York, 1952.
23. Bradley, O. C., "Topographical Anatomy of the Dog," 4th ed. Macmillan, New York, 1943.
24. Brambell, F. W. R., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), 3rd ed., Vol. I, p. 397. Longmans, Green, New York, 1956.
25. Bratt, H. M., Jr., and Burch, G. R., *North Am. Veterinarian* **33**, 541 (1952).
26. Brouha, L., *Cynécol. et Obstét.* **20**, 129 (1929).
27. Campbell, D. M., *Vet. Med.* **33**, 378 (1938).
28. (Contributed), *Vet. Record* **64**, 367 (1952).
29. Cotelin, E., *Brit. Vet. J.* **110**, 218 (1954).
30. Deumel, M., and Witzigmann, J., *Arch. wiss. prakt. Tierheilk.* **67**, 489 (1934).
31. DeVita, J., *J. Am. Vet. Med. Assoc.* **95**, 50 (1939).
32. Dott, N. M., *Quart. J. Exptl. Physiol.* **13**, 241 (1923).
33. Eckstein, P., and Zuckerman, S., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), 3rd ed., Vol. I, p. 543. Longmans, Green, New York, 1956.
34. Ellenberger, W., "Handbuch der Vergleichenden Mikroskopischen Anatomie der Haustiere," Vol. II. Paul Parey, Berlin, 1911.
35. Engle, E. T., *J. Mammal.* **27**, 78 (1916).
36. Erickson, S., *Nord. Veterinarmed.* **4**, 1078 (1952).
37. Evans, H. M., and Cole, H. H., "An Introduction to the Study of the Oestrous Cycle in the Dog." Mem. Univ. Calif., Vol. 9, No. 2, Univ. Calif. Press, Berkeley, Calif., 1911.
- 37a. Evans, H. M., Meyer, K., Simpson, M. E., and Reichert, F. L., "The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis." Mem. Univ. Calif., Vol. II, Sect. XII. Univ. Calif. Press, Berkeley, Calif., 1933.

- 3 Evans, H M, and Swezy, O, 'Ovogenesis and the Normal Follicular Cycle in Adult Mammals' *Mem Univ Calif*, Vol 9, No 3, Univ Calif Press, Berkeley, Calif, 1931
- 9 Frick, E J, *J Am Vet Med Assoc* 96, 76 (1940)
- 0 Friedman, M H, *J Am Vet Med Assoc* 130, 159 (1957)
- 1 Gardner, W U, *Endocrinology* 28, 53 (1941)
- 2 Gerlinger, H, These, Strasbourg, 1925
- 3 Cier, H T, *Vet Med* 49, 377 (1954)
- 4 Greenblatt, R B, and Pund, E R, *Southern Med J* 34, 730 (1941)
- 5 Griffiths, W F B, and Amoroso, E C, *Vet Record* 51, 1279 (1939)
- 6 Hammond, J, and Marshall, F H A, in *Marshall's Physiology of Reproduction* (A S Parkes, ed), 3rd ed, Vol II, p 793 Longmans, Green, New York, 1952
- 7 Hancock, J L, and Rowlands, I W, *Vet Record* 61, 771 (1949)
- 47a Hancock, J L, and Rowlands, I W, *Vet Record* 61, 771 (1949), also see R E Williams, in discussion
- 47b Hancock, J L, and Rowlands, I W, *Vet Record* 61, 771 (1949), also see G A Willis in general discussion
- 48 Harris, G W, "Neural Control of the Pituitary Gland" Edward Arnold, London, 1955
- 49 Harrop, A E, *Vet Record* 67, 494 (1955)
- 50 Hartman, C G, *Am J Anat* 37, 1 (1926)
- 51 Heape, W, *Quart J Microscop Sci* 44, 1 (1900)
- 52 Heape, W, *J Physiol (London)* 34, 1 (1905)
- 53 Helm, K, *Tierarztl Rundschau* 37, 671 (1931)
- 54 Houssay, B A, *Rev soc org biol* 11, 196 (1935)
- 55 Jackson W F, *Calif Vet* 7, 22 (1953)
- 56 Jonesheere F, *Arch biol (Paris)* 40, 357 (1930)
- 57 Kennedy, W P *J Anat* 58, 328 (1924)
- 58 Kunde, M M, D'Amour, F E, Carlson, A J, and Gustavson, R C, *Am J Physiol* 95, 630 (1930)
- 59 Kunde, M M, D'Amour, F E, Gustavson, R C, and Carlson A J, *Am J Physiol* 96, 677 (1931)
- 60 Lacroix, L J, in 'Canine Surgery' (K Mayer, J V Lacroix, and H P Hoskins, eds), 4th ed, p 472 American Veterinary Publications, Evanston, Illinois, 1957
- 61 Lang, J A, 'Fertility and Infertility in the Domestic Animals Aetiology, Diagnosis, and Treatment' Bullaire, Tindall & Cox, London, 1955
- 62 Leatherin, J H, *Endocrinology* 22 559 (1938)
- 63 Leatherin, J H, and Morrell, J A, *Endocrinology* 23 164 (1938)
- 63a Leatherin, J H, and Morrell, J A, *Endocrinology* 24, 149 (1939)
- 64 Leibold A A, *J Am Vet Med Assoc* 125 231 (1954)
- 65 Leonard, F P, Rickard, C G, and McEntee, K, in 'Canine Medicine' (H P Hoskins, J V Lacroix, and K Mayer, eds), p 110 American Veterinary Publications Evanston, Illinois, 1953
- 66 Laboulayre, M M, and Berthelot M, *Bull acad et France* 90 126 (1937)
- 67 Lyons, W R, Charkoff, I L, and Reichert, F L, *Proc Soc Exptl Biol Med* 31, 303 (1933)
- 68 Marshall, F H A, and Hahm F T, *Proc Roy Soc B* 89 346 (1917)

- 69 Marshall, F. H. A., and Jolly, W. A., *Phil. Trans. Roy. Soc. London* B198, 99 (1906).
- 70 Marshall, F. H. A., and Wood, W. A., *J. Physiol. (London)* 58, 74 (1923).
- 71 McDonald, L. E., McNutt, S. H., and Nichols, R. E., *Am. J. Vet. Research* 14, 539 (1953).
- 72 Meyer, R. K., and Saiki, S., *Proc. Soc. Exptl. Biol. Med.* 29, 301 (1931).
- 73 Mitchell, W. M., *Vet. Record* 49, 71 (1937).
- 74 Moss, W. P., *Vet. Record* 13, 33 (1933).
- 75 Mulligan, R. M., *J. Morphol.* 71, 431 (1942).
- 76 Mulligan, R. M., "Neoplasms of the Dog." Williams & Wilkins, Baltimore, Maryland, 1949.
- 77 Murray, G. H., and Eden, E. L., Jr., *Vet. Med.* 47, 467 (1952).
- 78 Newberry, W. E., and Gier, H. T., *Vet. Med.* 47, 390 (1952).
- 79 Papanicolaou, C. N., and Blau, N. F., *Anat. Record* 35, 47 (1927).
- 80 Parkes, A. S., *Physiol. Rev.* 25, 203 (1945).
- 81 Patten, B. M., "The Embryology of the Pig," 2nd ed., Blakiston Div., McGraw-Hill, New York, 1931.
- 82 Pearson, M., and Pearson, K., *Biometrika* 22, 309 (1931).
- 83 Pfaffner, J. J., Swingle, W. W., and Vars, H. M., *J. Biol. Chem.* 104, 701 (1934).
- 84 Raps, G., *Am. J. Vet. Research* 9, 61 (1948).
- 85 Reichert, F. L., *Endocrinology* 12, 451 (1928).
- 86 Riser, W. H., *J. Am. Vet. Med. Assoc.* 110, 86 (1947).
- 87 Roberts, S. J., "Veterinary Obstetrics and Genital Diseases." S. J. Roberts, Ithaca, New York, 1956.
- 88 Robson, J. M., and Henderson, W. R., *Proc. Roy. Soc.* B120, 1 (1938).
- 89 Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.* 79, 508 (1927).
- 90 Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.* 86, 20 (1928).
- 91 Rowlands, I. W., *Proc. Soc. Study Fertility No. 2*, 40 (1950).
- 92 Schotterer, A., *Anat. Anz.* 65, 177 (1928).
- 93 Scorgie, N. J., *Vet. Record* 51, 265 (1939).
- 94 Sisson, S., "The Anatomy of the Domestic Animals," 4th ed., Saunders, Philadelphia, Pennsylvania, 1953.
- 95 Smith, F., "A Manual of Veterinary Physiology," 5th ed. Baillière, Tindall & Cox, London, 1921.
- 96 Smith, H. A., and Jones, T. C., "Veterinary Pathology." Lea & Febiger, Philadelphia, Pennsylvania, 1957.
- 97 Spreull, J. S. A., *Vet. Record* 61, 579 (1949).
- 98 Stephenson, H., *Cornell Vet.* 20, 147 (1930).
- 99 Swingle, W. W., Parkins, W. M., Taylor, A. R., and Morell, J. A., *Proc. Soc. Exptl. Biol. Med.* 34, 94 (1936).
- 100 Tehver, J., *Rev. Vet. Estonienne* 10, 233 (1934).
- 101 Thorn, C. W., and Harrop, G. A., *Science* 86, 40 (1937).
- 102 Thorn, C. W., Nelson, K. R., and Thorn, D. W., *Endocrinology* 22, 155 (1938).
- 103 Trautmann, A., and Fiebiger, J., "Fundamentals of the Histology of Domestic Animals," rev. ed. Comstock, Ithaca, New York, 1952.
- 104 Tsukaguchi, H., and Okamoto, T., *Folia Anat. Japon.* 6, 663 (1928).
- 105 Turner, C. E., and Gomez, E. T., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 207*, 207 (1934).

106. Walton, A., *Proc. Soc. Study Fertility* No. 1, 40 (1949).
107. Whitney, L. F., *Chasc Mag.* 8, 4 (1927).
108. Whitney, L. F., *Vet. Med.* 31, 216 (1936).
109. Whitney, L. F., *Vet. Med.* 35, 182 (1940).
110. Whitney, L. F., *Science* 103, 654 (1946).
111. Whitney, L. F., *Vet. Med.* 42, 30 (1947).
112. Witschi, E., "Development of Vertebrates." Saunders, Philadelphia, Pennsylvania, 1956.
113. Wright, J. F., and Siebold, H. R., *J. Am. Vet. Med. Assoc.* 132, 258 (1958).
114. Wright, J. G., *Vet. Record* 14, 563 (1934).
115. Zuckerman, S., *Proc. Soc. Study Fertility* No. 4, 4 (1952).

CHAPTER 12

Fertilization and Development of the Egg

C. R. AUSTIN

	<i>Pa</i>
I. Maturation, Ovulation, and Transport of Eggs	41
A. Structure of Follicle and Oöcyte	41
B. Maturation of the Oöcyte	41
C. Mechanism of Ovulation	41
D. Time of Ovulation	41
E. Passage of Eggs to the Site of Fertilization	41
F. Fertile Life of Eggs	41
II. Transport of Spermatozoa	41
A. Deposition of Semen	41
B. Mechanism of Spermatozoon Transport	41
C. Rate of Spermatozoon Transport	41
D. Numbers of Spermatozoa	41
E. Fertile Life of Spermatozoa	41
III. Events Leading to Fertilization	41
A. Capacitation	41
B. Meeting of Egg and Spermatozoon	41
C. Reaction between Fertilizin and Antifertilizin	41
IV. Fertilization	41
A. Spermatozoon Penetration through the Cumulus Oöphorus	41
B. Spermatozoon Penetration through the Zona Pellucida	41
C. Entry of the Spermatozoon into the Vitellus	41
D. The Zona Reaction and the Block of Polyspermy	41
E. Activation	41
F. Pronucleus Formation, Growth, and Syngamy	41
G. The Function of the Pronuclei	41
H. Time Relations of Fertilization	41
I. Abnormalities of Fertilization	41
J. In Vitro Fertilization	41
V. Cleavage	41
A. Mechanisms of Cleavage	41
B. Early Differentiation of Cell Types	42
C. Cell Lineage	42
D. Rates of Cleavage and Volume Changes	42
E. Parthenogenesis	42
VI. Maintenance of the Early Embryo	42
A. Transport of the Embryo	42
B. Physiology of the Early Embryo	42
C. The Tubal and Uterine Environments	43
References	43

Most investigations on fertilization and associated phenomena in mammals have been made in the laboratory animals. The rodent eggs in particular, lend themselves well to detailed microscopic study while still alive, a property not shared by the eggs of domestic animals. A reasonably comprehensive account of present-day knowledge must therefore be based chiefly on what is known of laboratory animals. The course of events in maturation, fertilization, and the first cleavage of the egg is accordingly illustrated in this chapter by a series of diagrams drawn from observations on living rat eggs. Differences between rats and other animals are described when they are known and considered to be of sufficient importance. In writing this account, however, particular attention has been given to setting down the available data for domestic animals; where the text deals only with laboratory or nondomestic animals, it is because the equivalent information for domestic animals is lacking or seems unreliable.

Fuller details of subjects discussed in this chapter and more extensive lists of references are to be found in recent reviews (3, 4, 16, 17, 23, 35, 49, 61).

I. MATURATION, OVULATION, AND TRANSPORT OF EGGS

The eggs or ova develop in the ovary in *Graafian follicles* (see Fig. 1), which project as vesicular objects above the surface of the ovary in the later stages of their growth. Before fertilization can take place the egg must undergo a process of *maturation* or ripening. The release of the egg from the follicle—*ovulation*—occurs before, during, or after maturation, according to the species of mammal concerned. After ovulation, the egg is rapidly transferred to the Fallopian tube where, in the great majority of animals, fertilization begins.

A. Structure of Follicle and Oöcyte

When near full development, the follicle consists of an outer wall made up of two layers, the *theca externa* and the *theca interna*, lined internally by a broad zone of follicle cells, the *membrana granulosa*, containing a cavity or *antrum* filled with a quantity of free fluid, *liquor folliculi*. The egg, known at this stage as a *primary oöcyte*, is embedded in a part of the *membrana granulosa* that projects into the *liquor folliculi* and is referred to as the *cumulus oöphorus*. The *primary oöcyte* (Figs. 1 and 2a) consists of a mass of cytoplasm, the *cyte* which is limited by a cell membrane, the *vitelline membrane*, and is also invested by a thick transparent membrane, the *zona pellucida*. As the *zona pellucida* and the *vitellus* are in contact with each other the space

between them, the *perivitelline space*, is at this stage potential rather than real. Fine processes from certain of the immediately surrounding follicle cells traverse the *zona pellucida* and unite with the surface of the vitellus—they are thought to represent the means whereby the egg is nourished. Observations on the oocytes of kittens provide actual evidence that the transport of fats, and presumably of other nutrients also, is effected by these “nurse cells” (65). Somewhat eccentrically placed in the vitellus is the large spherical nucleus, the *germinal vesicle*, containing one or more nucleoli. The eggs of the placental mammals do not

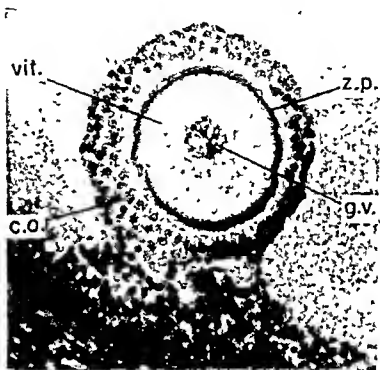


FIG. 1. The primary oocyte. Section of a large ovarian follicle in the cat, showing a primary oocyte. C.o. = cumulus oophorus, G.v. = germinal vesicle, Vit. = vitellus, Z.p. = zona pellucida. Magnification: $\times 200$. (Section by courtesy of E. C. Amoroso.)

differ much in size, in the majority of species, including the domestic animals and the rabbit, the over-all diameter of the egg is around 120–180 μ . ($1 \mu = 10^{-3}$ mm.). Rodent eggs vary between 75–100 μ in diameter.

B. Maturation of the Oocyte

Maturation chiefly involves nuclear structures. The germinal vesicle moves toward the surface of the egg, the nuclear membrane and nucleoli disappear, and the chromosomes condense into a compact and more easily demonstrable form (Fig. 2). The chromosomes then undergo two divisions, the point of special significance is that through these divisions the number of chromosomes originally present, the *diploid number*, is

reduced to half, the *haploid number*—a condition that characterizes the mature germ cells of both sexes. At each division, chromosomes are rejected from the vitellus in small masses of cytoplasm known as *polar bodies*. These chromosome divisions are referred to as *reduction, meiotic*, or *polar divisions*; it is the reduction in chromosome number that distinguishes the meiotic division from the mitotic division, which takes place when the fertilized egg or somatic cells divide. [For further information on meiosis see De Robertis *et al.* (26).] After the formation or *abstriction* of the first polar body, the egg is known as a *secondary oocyte*, and after the second polar body, as an *ootid*.

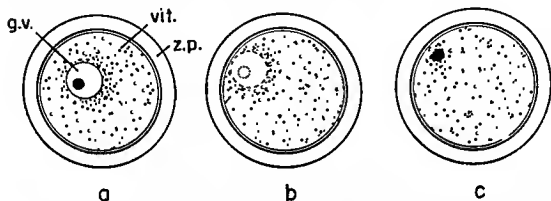


FIG. 2. Maturation of the rat oocyte—condensation of chromosomes. Germinal vesicle (G.v.) moves to the periphery, its structural details become vague and finally vanish. Chromosomes condense and gather together in the prophase of the first meiosis. Vit. = vitellus, Z.p. = zona pellucida.

Both reduction divisions take place in broadly the same way. Meiosis proceeds from *prophase*, through *metaphase* and *anaphase*, to *telophase* (Figs. 3, 4 and 5). In the later stages, a narrow granular zone, the *central body*, forms in the middle of the spindle. The spindle then rotates about one pole through roughly 90° , and, as this happens, a cleft develops in the surface of the vitellus and passes inward in the wake of the central body. The cleft eventually extends completely around the chromosome group at the pole of the spindle, thus cutting off a small mass of cytoplasm. Sometimes the chromosomes in the first polar body also undergo a second meiotic division and then the polar body itself may divide in two. In this way a total of three polar bodies may eventually be formed—a common occurrence in many invertebrate animals, but rare in mammals. Often the first polar body in the mammalian egg breaks up early, so that little or no sign of it may be seen after ovulation.

Coincident with the abstriction of the first polar body, the vitellus

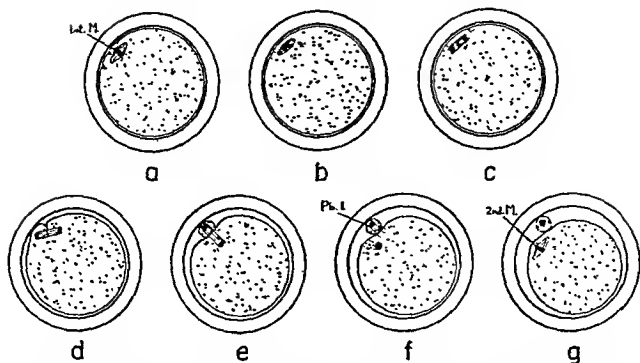


FIG. 3. Maturation of the rat oöcyte—first polar division. (a), (b), and (c): Diagrams showing the first meiotic spindle (1st M.) in metaphase, anaphase, and telophase, respectively. (d) and (e): Rotation of the spindle and development of the cytoplasmic fissure. (f): Abstriction of the first polar body (Pb. 1.) is completed. The vitellus has shrunk slightly so that there is now a more distinct perivitelline space. (g): State of the egg at ovulation: the second meiotic spindle (2nd M.) is in metaphase, and the first polar body is usually lacking owing to its early degeneration.

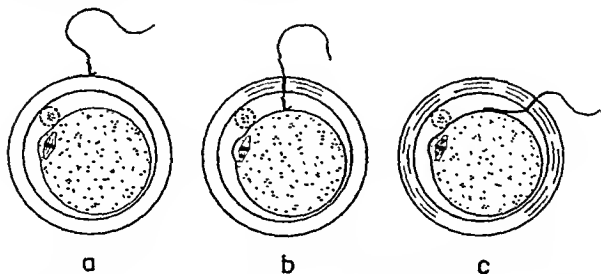


FIG. 4. Spermatozoon entry into the rat egg. (a): Spermatozoon has become attached to the surface of the zona pellucida; the acrosome is removed at or just before this event. (b): Spermatozoon head has passed through the zona pellucida and now adheres to the surface of the vitellus. As a result, the block to polyspermy has been evoked (represented by thickened line limiting the vitellus) and the zona reaction has begun (represented by shading of the zona pellucida). (c): Spermatozoon head lies for a while flat upon the vitelline surface before being absorbed into the vitellus. Zona reaction has travelled further round the zona pellucida. From Austin and Bishop (4).

undergoes a slight shrinkage; as a result, a true though small perivitelline space is formed. A second contraction, accompanied by a further enlargement of the perivitelline space, occurs at about the time of abstriction of the second polar body.

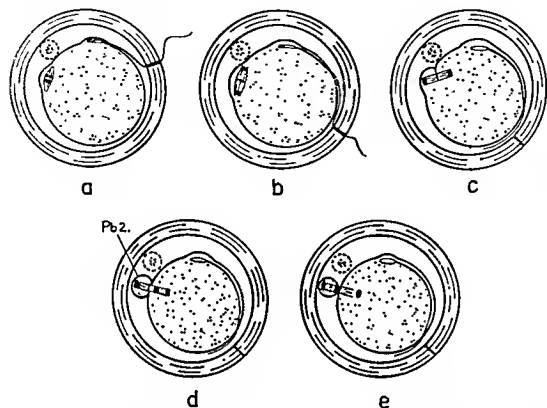


FIG. 5. Maturation of the rat oocyte—second polar division—early fertilization. (a): As the spermatozoon head passes into the vitellus, the second meiosis proceeds to anaphase. In this and the other diagrams of this figure, the spermatozoon head is shown undergoing the initial phase of its transformation into a male pronucleus. (b): Meiosis in telophase. (c), (d), and (e): Rotation of the spindle and abstriction of the second polar body (Pb 2). From Austin and Bishop (4).

C. Mechanism of Ovulation

Shortly before ovulation, follicular growth passes through a terminal phase in which the volume increases at a rapidly accelerating rate and which culminates in ovulation. A small elevation makes its appearance on the surface of the projecting follicle; soon its thin wall breaks down, leaving an aperture through which the follicular contents stream out. Emission of this material may be assisted to a small degree by contraction of the smooth muscle fibers of the theca externa, although generally the follicle undergoes little diminution in size. It is possible that a similar muscular contraction, aided by rapid secretion of liquor folliculi, may

raise intrafollicular pressure before ovulation and that, as a result, the act of ovulation may occur explosively. However, rupture under pressure is almost certainly not the essential mechanism of follicular opening and, in most instances, ovulation is a gradual process, depending as much upon progressive changes in the structure of the follicle wall as upon the effect of internal pressure (19). When the egg leaves the follicle, it is still surrounded by the cumulus oophorus.

D Time of Ovulation

In general, ovulation occurs near the end of estrus, but the precise time relation differs in different species. Estrus in the mare lasts 7 days on the average, though it may be as short as 3 days or as long as 30 days, irrespective of length of estrus, ovulation mostly occurs 1 to 2 days before its close. The time of ovulation in the cow is unusual—about 12 to 15 hours after the end of the 18 hour estrus. In other ruminants, the ewe and the goat, the eggs are ovulated a few hours before the end of estrus, which lasts an average of 24 and 40 hours, respectively. The sow's eggs are ovulated about 36 hours after the start of the 40- to 60 hour estrus. The bitch is another animal with a prolonged estrus, lasting about 9 days, and, uniquely, ovulation occurs on the 1st or 2nd day after the beginning of sexual receptivity. In the cat (as in the ferret, rabbit, and a few other species) ovulation is induced by the act of coitus. Coitus in the cat may take place at any time during the 3-day estrus and is followed by ovulation 24 to 30 hours later (25) or, sometimes, 40 to 54 hours later (37).

Most mammalian eggs are ovulated when the second maturation division has reached metaphase (Fig 3g). In the dog and probably the horse, however, the egg is ovulated as a primary oocyte and passes through its maturation in the Fallopian tube.

E Passage of Eggs to the Site of Fertilization

The site of fertilization in the domestic animals is in the *ampulla* of the Fallopian tube. This is true for most mammals, the only notable exceptions being the ferret and the short-tailed shrew, wherein fertilization is said to begin in the ovarian bursa, and the tenrecs of Madagascar, wherein spermatozoa enter the follicles and penetrate the eggs before ovulation.

At ovulation, the egg and its cumulus oophorus are liberated onto the surface of the ovary and very shortly pass into the *infundibulum*, the specialized ovarian end of the Fallopian tube. The means whereby this transfer is effected are not yet clearly understood. Observations in the rabbit, in which the *infundibulum* is large and funnel-shaped, indi-

cate that this structure plays an active role by "embracing" the ovary at the time of ovulation and taking up the eggs with a to-and-fro movement (31). Cinematographic records prepared by R. J. Blandau show that in the rat the eggs, suspended in the bursa fluid, drift from the ovary into the opening of the infundibulum, which in this species is not dilated. Presumably the drift is attributable to currents set up by the action of cilia lining the internal surface of the Fallopian tube. Though ciliary currents may assist the process in the domestic animals, the possession of funnel-shaped, fimbriated infundibula, particularly by the larger members of the group, suggests that the mechanism would more closely resemble that described for the rabbit. Once within the Fallopian tube, contractions of the tubal wall probably play a part, but ciliary currents may well be of chief importance, for the whole tube is lined with cilia and their beat is uniformly directed toward the uterus.

Sometimes, an egg fails to pass into the Fallopian tube on its own side and, by some means, traverses the peritoneal cavity and enters the contralateral tube. This phenomenon is called *external migration* and has been reported in human subjects and the rabbit.

F. Fertile Life of Eggs

Precise data are difficult to obtain, but the indications are that the eggs of the domestic animals remain fully viable for 12 to 24 hours after ovulation and perhaps a little longer, except in the dog, in which the fertile life of the egg probably exceeds 4 days and may possibly be as long as 8 days. Loss of viability is not sudden—aging eggs may be able to undergo apparently normal fertilization, but give rise to embryos that die before birth. With further deterioration, fertilization becomes abnormal or fails altogether.

II. TRANSPORT OF SPERMATOZOA

A. Deposition of Semen

At coitus, semen is passed into the female tract, but the volume of the ejaculate and the site of its deposition vary. In the horse, the ejaculate amounts to about 75–150 ml. and the semen is projected into the cranial end of the vagina and through the relaxed cervical canal into the uterus. In the pig, the ejaculate is even more voluminous, being usually about 125–500 ml.; it is slowly propelled through the vagina and cervix and into the uterus during the prolonged coitus. In the ox and sheep, the volumes of ejaculates are about 5 and 1 ml., respectively, and the semen is deposited in the cranial part of the vagina and on the cervix. In the dog and cat the ejaculates measure about 7.0 and 0.5 ml., respectively, and are deposited in the vagina.

B. Mechanism of Spermatozoon Transport

To varying degrees in different animals, transport of spermatozoa in the female tract occurs during coitus and while they are suspended in the seminal plasma. Admixture with the secretions of the female tract soon takes place, however, and as the spermatozoa approach the site of fertilization female secretions alone constitute the suspending medium.

In the horse, it has been demonstrated that, as a result of the female orgasm, negative pressure develops in the uterine lumen (41); this would no doubt facilitate the rapid passage of semen into the uterus. In both horse and pig, the ejaculate has been found to pass as far as the uterotubal junction (40). Radiopaque fluid, injected experimentally into the uterus of the estrous cow, has been observed to move rapidly to the uterotubal junction; when oxytocin was administered to simulate the effect of coitus, the fluid was found to enter the Fallopian tubes within $2\frac{1}{2}$ minutes of its injection (50).

These rapid movements of material within the uterine lumen are attributable to the contractions of the uterine wall. The contractions are powerful during estrus and are strongly augmented when the orgasm is evoked (56, 57, 58); they have the effect of churning up the contents of the organ. Similar forces are brought to bear upon the spermatozoa when they reach the Fallopian tube.

Thus, spermatozoa are transported, perhaps a little by their own motility, but very largely by the process of being passively mixed with the fluid contents of the tract. As a result, the distribution of spermatozoa in any one region tends to become progressively more uniform. Uniformity of distribution throughout the entire tract, however, is not even remotely achieved because of the barriers presented by the cervix uteri, the uterotubal junction, and the isthmus of the Fallopian tube. Consequently, where ejaculation is into the vagina, the concentration of spermatozoa remains much higher in the vagina than in the uterus, and very much higher than in the Fallopian tube. The over-all effect is that very small numbers of spermatozoa are rapidly brought to the site of fertilization.

C. Rate of Spermatozoon Transport

Transport of spermatozoa to the ampulla of the Fallopian tube is remarkably rapid: times of 15 minutes or less have been reported for the cow, ewe, and bitch. In the cow, dead spermatozoa were transported as quickly as living ones (59), which serves to emphasize the small contribution that the cells' swimming movements make toward their transport.

Under normal circumstances, and where full opportunity exists, coitus takes place early in the heat period, so that spermatozoa reach the site

of fertilization several hours before the eggs. The termination of the period of sexual receptivity normally precludes the possibility of unduly delayed coitus and, consequently, the possibility that the eggs would have to wait long for the spermatozoa to arrive. In the cat, coitus-induced ovulation makes it even more certain that if conditions are normal spermatozoa will be at the site of fertilization well before the eggs. With artificial insemination, however, unless due care is taken, there is a risk that the natural time relations will be disturbed and fertility thus reduced.

D. Numbers of Spermatozoa

Average figures for the total number of spermatozoa in a single ejaculate may be given as follows: stallion, 6000×10^6 ; bull, 3000×10^6 ; ram, 800×10^6 ; boar, $20,000 \times 10^6$; dog, $35,000 \times 10^6$ (27); cat, 800×10^6 (E. C. Amoroso, personal communication). These are enormous numbers and represent in each instance very many more spermatozoa than are necessary for normal fertility. If, for example, an average bull ejaculate is suitably diluted and used for the artificial insemination of about 500 cows, the great majority of them are likely to become pregnant.

The numbers of spermatozoa reaching the more ovarian regions of the tract progressively diminish; in the ewe, the average numbers in the uterus and Fallopian tubes some hours after mating were found to be only 76,000 and 6,500, respectively (62). The number at the actual site of fertilization is evidently less than 100 in the rat and mouse, and less than 1000 in the rabbit and ewe (3).

E. Fertile Life of Spermatozoa

In general, spermatozoa, like eggs, are incapable of retaining full viability and fertility in the female tract for much longer than 24 hours. The longest period in most animals has been found to be 2 to 3 days, although in the mare it may be as long as 5 days. A remarkable exception to the general rule is shown by some species of bats, in which coitus takes place in the autumn, and ovulation and fertilization do not occur until the following spring—an interval that may be as long as 5 months. Spermatozoa, like eggs, probably lose the ability to give rise to normal young before they cease to be capable of taking part in fertilization.

III. EVENTS LEADING TO FERTILIZATION

A. Capacitation

The arrival of spermatozoa at the site of fertilization before the eggs suggests that spermatozoa are not capable of participating in fertiliza-

tion immediately upon entering the female tract, but must reside there for a period to develop this capacity. In recent years, observations on rats and rabbits have consistently supported this idea (3). Briefly, the evidence shows that the spermatozoa are required to undergo some form of physiological preparation, referred to as *capacitation*, within the female tract to fit them for the task of penetrating the zona pellucida of the eggs. Capacitation appears to involve a change in the properties of the *acrosome* which leads to its detachment when the spermatozoon makes contact with the zona pellucida (5). (The term "acrosome" as used here is equivalent to "acrosomic system" in Volume II, Chapter I.) Capacitation takes about 2 hours in the rat and 4 hours in the rabbit.

B. Meeting of Egg and Spermatozoon

The spermatozoa of certain plants (ferns, mosses, etc.) are known to be attracted to the egg cells by chemical substances secreted by them or by neighboring cells (49). Attraction of the male gamete by the female in this way is an example of *chemotaxis* and undoubtedly aids in assuring their union. Despite numerous investigations on animal gametes, mainly those of invertebrate animals, no unequivocal evidence has yet been produced that chemotaxis between eggs and spermatozoa operates in the animal kingdom.

In mammals, the chances of the meeting of eggs and spermatozoa are the resultant of several interacting influences and conditions. Basically, they depend upon the speed of movement of the spermatozoa, the concentration of spermatozoa about the eggs, and the surface area of the eggs. Concentration of spermatozoa can probably be taken as the chief variable. Available data suggest that the chances of fertilization are normally of the same order in all species: although more spermatozoa are known to reach the site of fertilization in sheep and rabbits than in rats and mice, the former animals have much larger ampullae and consequently much more space for the spermatozoa to occupy. Actual estimates, based on several lines of evidence, have indeed shown that the chances are much the same in the rat and the rabbit (7).

In those animals in which it persists, the cumulus mass about the eggs may well facilitate meeting of eggs and spermatozoa by providing a larger target area, and the radially arranged follicle cells may help to orientate spermatozoa toward the egg.

A special significance of the small number of spermatozoa at the site of fertilization deserves emphasis. The participation of more than one spermatozoon in fertilization (*polyspermy*) is a pathological occurrence in mammals and almost certainly leads to early death of the embryo

(see p. 417). As eggs themselves have imperfect, direct protection against the penetration of more than one spermatozoon, it is important for the survival of the species that the chances of fertilization should not surpass a certain upper limit. It is, of course, equally important that they should not fall below a certain limit, if fertility is to be maintained. An essential function of the female genital tract is, therefore, to control the transport of spermatozoa in order that the number reaching the site of fertilization will be sufficient to provide good chances of fertilization for all eggs without being so large as to cause serious risk of polyspermy.

C. Reaction between Fertilizin and Antifertilizin

It was shown many years ago by Lillie (38) that, when sea urchin spermatozoa are placed in sea water in which eggs have been standing, the spermatozoa are strongly agglutinated. This effect he attributed to an agent, which he called *fertilizin*, that diffuses from the eggs into the surrounding medium. The constituent of the spermatozoon with which fertilizin reacts is termed *antifertilizin*; it can readily be extracted from the spermatozoa. The fertilizin-antifertilizin reaction is believed to have an important function in fertilization, in making possible the attachment of the spermatozoon to the egg, and also perhaps the subsequent passage of the spermatozoon into the egg. In addition, the species specificity of the reaction tends to prevent cross-fertilization by spermatozoa of other species (55).

Recently, evidence has been brought forward to show that substances analogous to fertilizin and antifertilizin exist in mammalian germ cells (15). Rabbit, mouse, bull, and human spermatozoa were found to be agglutinated in the presence of eggs, more especially (with the first three species) when homologous eggs were used. It is suggested that, as in invertebrates, the egg fertilizin reacts with the spermatozoon antifertilizin, ensuring attachment of the spermatozoon and providing a mechanism for its penetration into the egg.

IV. FERTILIZATION

Fertilization involves (a) the penetration of the spermatozoon into the egg; (b) the formation of a spermatozoon nucleus, the *male pronucleus*, and an egg nucleus, the *female pronucleus*; (c) the growth and development of the pronuclei; (d) the replacement of the pronuclei by chromosome groups; and, finally, (e) the union of the two chromosome groups. The essential feature of fertilization lies in the mingling of paternal and maternal chromosomes: by this act, chromosomes from two different sources are brought together to constitute the genetic material

of the new individual and the diploid chromosome number is restored. In addition, there are two other consequences of spermatozoon entry: the egg is stimulated to development, i.e., it is *activated*, and through the medium of the spermatozoon tail, paternal cytoplasmic elements are contributed to the embryo.

A. Spermatozoon Penetration through the Cumulus Oöphorus

The eggs arrive in the Fallopian tube surrounded by the cumulus oöphorus, which consists of a large number of follicle cells embedded in a jellylike matrix composed of a mucoprotein known as *hyaluronic acid*. Immediately about the egg, the cells are more densely packed and, particularly in dog, cat, and rabbit eggs, this region presents a distinctive appearance and is often termed the *corona radiata*. At least some of the coronal cells evidently retain the direct attachment to the egg that they exhibited while in the follicle. In the mare, cow, and ewe, the cumulus breaks down early so that tubal eggs are generally recovered with few or no follicle cells attached.

The spermatozoon has been shown to carry an enzyme that is capable of dissolving hyaluronic acid and which is therefore named *hyaluronidase*. The enzyme can easily be extracted from spermatozoa. When such an extract is added *in vitro* to eggs, with their cumulus masses, the matrix dissolves and the follicle cells fall away. Eggs other than those of the dog, cat, and rabbit are thus stripped of adherent cumulus; in these three species, however, the *corona radiata* remains intact, although any hyaluronic acid between the cells has presumably passed into solution.

Observations in the laboratory animals have shown that *in vivo* spermatozoa enter the eggs without the cumulus suffering noticeable disintegration. It is believed that the spermatozoa, with the aid of hyaluronidase, individually digest paths for themselves through the matrix of the cumulus and between the coronal cells. The cumulus is broken down later, during the course of fertilization. This appears to be brought about partly by hyaluronidase diffusing from spermatozoa at the site of fertilization, partly through an autolytic process, and partly through the operation of some as yet unidentified factor in the Fallopian tube. Autolysis and the "tubal factor" may be jointly responsible for the early disappearance of the cumulus mass from about the eggs of the horse and the ruminants. In dog and cat eggs, on the other hand, the *corona* persists for much longer, even throughout early cleavage (see Fig. 9).

B. Spermatozoon Penetration through the Zona Pellucida

Less is known of the means by which the spermatozoon is able to penetrate the zona pellucida. A narrow slit that the spermatozoon leaves

in the zona after it has passed through has been identified in some rodent eggs; this may mean that the spermatozoon carries an enzyme, provisionally called the *zona lysin*, that acts upon the substance of the zona, softening it, as it were, thus permitting the spermatozoon to make its way through. It is suggested that the lysin is carried upon a modified part of the nuclear membrane of the spermatozoon head that is called the *perforatorium*. This is the region that is exposed by the removal of the acrosome, which occurs when the spermatozoon reaches the zona pellucida (5).

The principal significance of spermatozoon motility is probably that it supplies the motive force that propels the cell through cumulus oöphorus and zona pellucida.

C. Entry of the Spermatozoon into the Vitellus

Passage through the zona pellucida is rapidly accomplished and the spermatozoon head then projects into the perivitelline space and makes contact with the surface of the vitellus (Fig. 4). Contact appears to evoke a response from the vitelline cytoplasm so that attachment is formed with the spermatozoon head. After a pause, the head, with tail still attached, is taken into the vitellus (Fig. 5), much as a food particle is engulfed by an amoeba. The pause before actual entry into the vitellus is evidently quite appreciable—it was found to be about $\frac{1}{2}$ hour in some rodent eggs. For a while after spermatozoon entry, the surface of the vitellus is elevated above the head in a manner that is reminiscent of the *fertilization cone* of some invertebrate eggs. In the great majority of animals, both vertebrate and invertebrate, the entire spermatozoon tail passes into the vitellus with the head; exceptions among mammals include the field vole and Chinese hamster.

In most mammalian eggs the metaphase of the second maturation division is evident at ovulation (Fig. 3g), and meiosis does not normally continue until a spermatozoon head becomes attached to the surface of the vitellus. Entry of the spermatozoon is therefore accomplished during the early phase of the resumption of meiosis (Fig. 5a). Spermatozoon penetration in the dog commonly occurs while the egg is still a primary oöcyte; the spermatozoon head then lies quiescent in the vitellus, showing little change until abstriction of the second polar body is completed.

D. The Zona Reaction and the Block to Polyspermy

Mention was made earlier of the fact that polyspermy is probably lethal to the embryo and that the significance of the very small numbers of spermatozoa at the site of fertilization was that the chances of fertili-

zation were thereby reduced. Complementary to this mechanism is the egg's own defense system, which tends to prevent the entry of more than one spermatozoon. Defense is vested in two membranes, namely, the zona pellucida and the vitelline membrane. In most mammals, it seems, both membranes are capable of undergoing a change after the entry of the first spermatozoon—a change that makes them impermeable to subsequent spermatozoa. The alteration in the zona pellucida is termed the *zona reaction* and that in the vitelline membrane the *block to polyspermy*. Evidence indicates that both are evoked by the attachment of the spermatozoon head to the vitelline surface (Fig. 4, the increased thickness of the line limiting the vitellus is intended to represent the occurrence of the block to polyspermy and the shading in the zona, the *zona reaction*). From the point of attachment of the spermatozoon the block to polyspermy passes around the cortex of the egg as a propagated change. In the sea urchin it takes only about one minute for the block to become complete over the whole egg surface—there is, as yet, no known way of determining the corresponding time for mammalian eggs. The *zona reaction* is thought to be brought about by some substance that is released from the surface of the vitellus and diffuses across the perivitelline space (8). The release of this agent seems to be propagated over the egg surface like the block to polyspermy, but the *zona reaction* itself may be comparatively slow, taking, in the rat, something between 10 minutes and 2 hours to reach completion (18).

Rabbit eggs appear to be unique in that, though they can exhibit a block to polyspermy, they do not seem capable of developing a *zona reaction*; spermatozoa continue for several hours to pass through the zona and accumulate in the perivitelline space (Fig. 7a). Spermatozoa that pass through the zona pellucida but are excluded from the vitellus by the block to polyspermy are known as *supplementary spermatozoa*; they are not known to have any role in fertilization or in the development of the embryo. Rabbit eggs may have as many as 200 odd supplementary spermatozoa. Rat and mouse eggs, on the other hand, give evidence of the capacity to develop both changes: they often have one or two supplementary spermatozoa, but seldom more; the frequency distribution of such spermatozoa is very different to what would be expected by chance. The eggs of several other animals, including the domestic animals, have not been observed with supplementary spermatozoa, although numerous spermatozoa commonly adhere to the outside of the zona pellucida. From these facts it is inferred that these eggs have a very rapid *zona reaction* and that this is mainly responsible for their protection against polyspermy.

E. Activation

Attachment of the spermatozoon head to the surface of the vitellus is also in a change of a different nature, namely, an awakening of the egg from a dormant state. This stimulus to development is known as *activation* and the details of its mechanism have been sought for many years. From work on invertebrates it has long been known that many agents of a chemical, physical, or mechanical nature can activate eggs, including heat and cold, acids and alkalis, hyper- and hypotonic solutions, radiations, alkaloids, fat solvents, mechanical agitation, and electric currents. Activation may also occur apparently spontaneously, without the participation of any identifiable external factor. However, the particular property of the spermatozoon that is responsible for activation remains a mystery. Specific activation (by the spermatozoon), leads normally to pronucleus formation and development. Nonspecific activation (by some other means), on the other hand, is rarely followed by these processes in mammals—when it is, the phenomenon is regarded as the initiation of *parthenogenesis* (see Section V, E).

F. Pronucleus Formation, Growth, and Syngamy

Soon after the spermatozoon head enters the vitellus, it begins to swell and, by subtle changes involving the production of nucleoli and a surrounding nuclear membrane, it is converted into a male pronucleus. Simultaneously, the second polar body is abstricted and the group of chromosomes remaining within the vitellus becomes transformed into the female pronucleus (Fig. 6). The pronuclei increase progressively in volume until they are about twenty times their original size, and move toward each other through the cytoplasm, so that when fully grown they are in close contact. Then, quite suddenly, they diminish in volume and finally fade out altogether, giving place to two chromosome groups. In their turn, these chromosome groups move together and form a single group which represents the prophase of the first cleavage division. The terminal stages, from contact between the pronuclei to union of the two chromosome groups, is known as *syngamy*. Fertilization is now complete.

Figure 6 shows the appearance presented by rat eggs during fertilization. In this species the relatively large pronuclei have several large nucleoli; the volume of the male pronucleus is about two and a half times that of the female. Pronuclei in the eggs of domestic animals and the rabbit, on the other hand, are relatively small and the nucleoli are few and small; in addition, there is not so much disparity in size between male and female pronuclei (Fig. 7). In most respects, however, the changes exhibited by rat eggs during fertilization may reasonably be considered representative of those of mammalian eggs in general.

G. The Function of the Pronuclei

It seems likely that pronuclear function includes the synthesis of deoxyribonucleic acid (DNA) (24). This substance is the characteristic constituent of chromosomes and hence is a regular component of nuclei. Deoxyribonucleic acid is believed to be the essential hereditary material, bearing in its molecular configuration the genetic information that is passed on from the spermatozoon and egg nuclei to the nuclei of the early embryo, and thence to all the cells of the new individual. In any

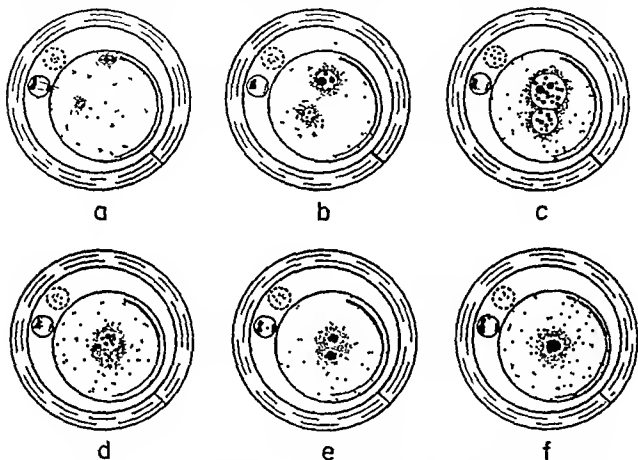


FIG. 6. Pronuclear development and syngamy in the rat egg. Diagrams showing the growth of male and female pronuclei, and their conjugation, diminution, and final replacement by chromosome groups which come together in the prophase of the first mitosis. From Austin and Bishop (4).

one species, the absolute amount of DNA in a nucleus is proportional to the chromosome number. Thus, the spermatozoon head and the nucleus of the fully matured egg each contain only half the DNA complement of a somatic nucleus, since they each have only half the number of chromosomes. When the fertilized egg undergoes its first division, two cells are formed, in each of which there is a nucleus with the same amount of DNA as a somatic nucleus. Hence the need for synthesis of extra DNA—not only by the pronuclei, of course, but also by the nuclei of subsequent embryonic cells.

Sea urchin eggs are said to contain sufficient stores of DNA in their cytoplasm for about sixteen cleavage nuclei. Sea urchin pronuclei, therefore, may not need to synthesize DNA. Since no cytoplasmic DNA has been detected in mammalian eggs, the account just given of mam-

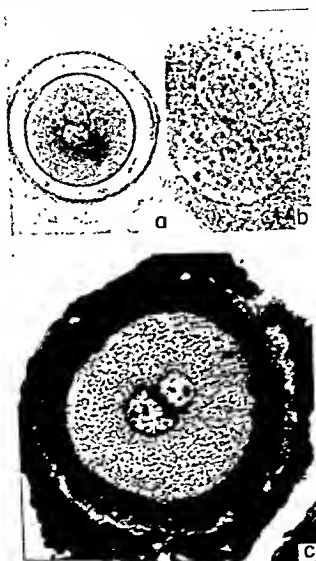


FIG. 7. Pronuclei. (a) and (b): Pronuclei in living rabbit eggs, as seen by phase-contrast microscopy. Magnifications: $\times 200$ and 600 , respectively. (c): Pronuclei in a section of a fixed dog egg. Magnification: $\times 350$. Owing to the texture of the cytoplasm, pronuclei are almost impossible to discern in the living eggs of domestic animals; it is reasonable to suppose, however, that their appearance would resemble that of the rabbit pronuclei. (Fig. 7c by courtesy of E. C. Amoroso.)

malian pronuclear function is reasonable. Another function, however, has been suggested for mammalian pronuclei, namely, that they may be involved in the production of patterns or "templates" in readiness for the protein synthesis that will go on during embryonic growth.

H Time Relations of Fertilization

In the rabbit, the spermatozoa reach the site of fertilization 6 to 7 hours before the eggs, and proceed to penetrate the eggs during the 1 or 2 hours after ovulation. Pronuclei are formed about $1\frac{1}{2}$ hours later. The first cleavage of the fertilized egg takes place 11 to 14 hours after ovulation. The pronuclear life span is thus about 8 to 10 hours. Similar information is available for some other laboratory animals, but data on domestic animals are extremely fragmentary. Moreover, in most instances the times recorded refer to coitus as the starting point, obviously, this will lead to the introduction of relatively large errors, as the interval between coitus and ovulation in the domestic animals (except the cat) is by no means constant. The more useful figures that have been published so far are as follows: spermatozoon penetration takes place 2 to 3 days after coitus in the cat (i.e., about 1 day after ovulation). Pronuclei are formed between 11 and 39 hours after ovulation in the cow, and less than 36 hours after coitus in the ewe. The fertilized egg undergoes its first cleavage about 24 hours after ovulation in the pony mare, 30 hours after coitus in the goat, 38 to 39 hours after coitus in the ewe, and 50 hours after coitus in the sow.

I Abnormalities of Fertilization

Experience with laboratory animals indicates that, under favorable circumstances, accidents rarely occur in fertilization, in spite of the complexity of the process. Abnormalities are likely to be induced, however, by anything that disturbs the normality of either gamete, such as aging of the gametes, elevation of temperature, X irradiation, and administration of certain toxic substances. The chief irregularities in fertilization that yet permit some degree of embryonic development involve incomplete maturation of the egg, polyspermy, and the failure of either spermatozoon nucleus or egg nucleus to develop.

Incomplete maturation of the egg implies that abstriction of either or both polar bodies fails—meiosis proceeds to anaphase or telophase, but both chromosome groups remain within the vitellus. As a result, the fertilized egg will have too many sets of chromosomes and the embryo will be *polyploid*. Failure to abstrict one polar body leads to *triploidy* after fertilization, and failure to abstrict both polar bodies to *pentaploidy*. Except for some rather dubious reports for the rabbit and pig, there is as yet no record of the birth and survival to maturity of a polyploid mammal (11).

Polyspermy results when two or sometimes three spermatozoa, having evidently approached the egg almost simultaneously, succeed in gaining

entrance together and participate in fertilization. Since neither the zona reaction nor the block to polyspermy can be instantaneous, such a risk inevitably exists. Both, or all three, fertilizing spermatozoa form pronuclei and these later undergo syngamy with the female pronucleus. The additional fertilizing spermatozoa and male pronuclei are referred to as *supernumerary*. In the rat, at least, fertilization by two spermatozoa gives rise to a triploid embryo, which can certainly develop to the 8-cell stage and possibly a little further (6). It may reasonably be assumed, however, that, as with other kinds of mammalian polyploids, polyspermic embryos probably do not survive to term. Trinucleate, probably polyspermic eggs have been recovered from cats at an incidence of about 3% (60).

If an embryo develops from an egg in which fertilization began normally but was vitiated by the failure of the male pronucleus, the process is termed *gynogenesis*. If, on the other hand, it was the female pronucleus that failed, the process is called *androgenesis*. Once again, the primary effect of these anomalies on the embryo is a disturbance of chromosome number: both gynogenetic and androgenetic embryos have half the normal chromosome complement, or in other words they are *haploid*. Like polyploid embryos, haploid embryos are most unlikely to develop far.

J. In Vitro Fertilization

It has long been an ambition of biologists to find conditions that would permit the fertilization of the mammalian egg to proceed under direct observation *in vitro*, but the process of spermatozoon penetration seems to depend upon a highly specific environment, the components of which have yet to be adequately defined. Nevertheless, numerous claims have been made that the necessary conditions had been experimentally achieved and that fertilization had taken place. None of these is quite convincing, however, because of failure to establish satisfactorily the occurrence of spermatozoon penetration into the vitellus or to exclude the possibility of nonspecific activation of the egg.

V. CLEAVAGE:

The conclusion of fertilization marks the genesis of the *zygote* or early embryo. Initially, the salient feature of development is a special form of cell division known as *cleavage*, during which the protoplasmic mass of the embryo is progressively divided until it composes a large number of cells. Cleavage involves no gain and, indeed, some loss of total protoplasmic mass; it continues until the cells constituting the embryo are of a size that is normal for most adult tissues of the animal

cerned. Cleavage then ceases; cell division continues but is now associated with increase in total mass, that is to say with the growth of the embryo.

A. Mechanisms of Cleavage

Fertilization ends with the union of the paternal and maternal chromosome groups. The chromosomes become arranged on the first cleavage spindle and mitosis proceeds through metaphase and anaphase to

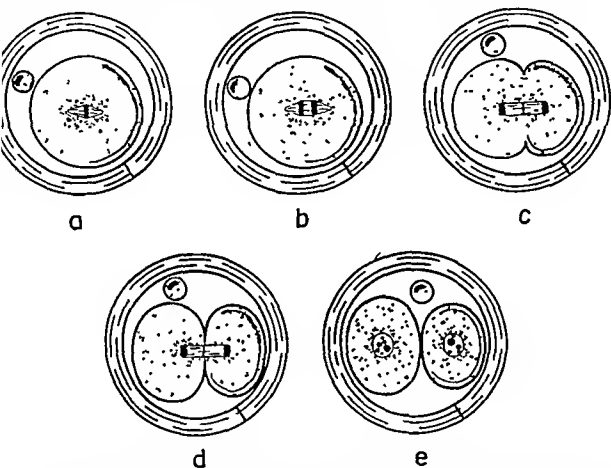


FIG 8 The first cleavage of the rat egg. (a): Cleavage spindle develops in the center of the egg. (b) and (c): Mitosis proceeds through anaphase to telophase. The vitellus elongates and the cytoplasmic surface around the equator of the egg dips inward. (d): Cleavage furrow has nearly completed division of the cytoplasm. (e): Following cytoplasmic division, nuclei reform.

telophase (Fig. 8). The cytoplasm then divides into two, usually unequal, cells that are called *blastomeres*. Cytoplasmic division begins with a dipping-in of the surface which is believed to be attributable, not to a ring of contracting cytoplasm, but to expansion of the general cell surface induced in the polar regions by substances emanating from the chromosome groups (42). Consistently, the egg becomes somewhat elongated before actual cleavage starts. The plane of cleavage passes through the centers of the areas that were occupied by the male and female pronuclei at the beginning of syngamy. When cytoplasmic divi-

sion is complete, the chromosomes resume the extended form, nucleoli develop, and so the zygote nuclei make their appearance (Figs. 8e and 9b).

The second cleavage division usually occurs first in the larger blastomere—the nucleoli disappear, chromosomes condense, mitosis proceeds, the cytoplasm divides, and nuclei reform. Thus a 3-cell embryo is

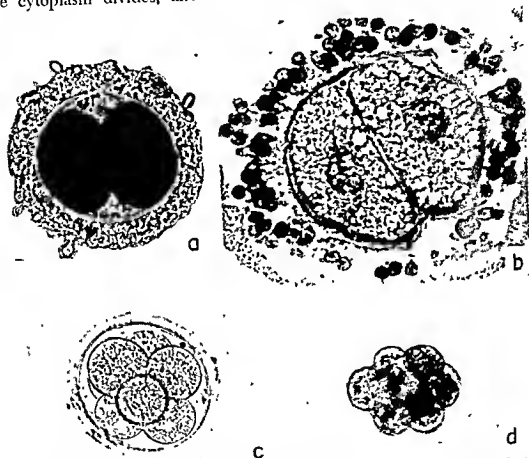


FIG. 9. Early cleavage embryos. (a) Living dog embryo at the 2-cell stage and (b) section of a 2-cell cat embryo. Both showing adherent follicle cells. Magnifications: $\times 225$ and 350 , respectively. (c): Living 8-cell embryo of sheep and (d) living 8-cell embryo of pig. Magnifications: $\times 200$ and 180 , respectively. (By courtesy of E. C. Amoroso.)

formed. The smaller of the first two blastomeres then divides in the same way, to give a 4-cell embryo. Completion of the third cleavage division in all these four blastomeres yields the 8-cell embryo (Figs. 9c and d); since divisions are seldom exactly synchronous, however, 5-, 6-, and 7-cell embryos may be seen earlier. After the fourth series of cleavage divisions, the embryo consists of 16 cells, and after the fifth, of 32 cells. Partly because of the restricted space within the zona pellucida and

partly because the planes of the spindles of successive cleavage divisions tend to be orientated approximately at right angles to those of the previous divisions, the cells form a compact group known as a *morula* (Figs. 10, 12a and 13a). The constituent cells of the morula are not all

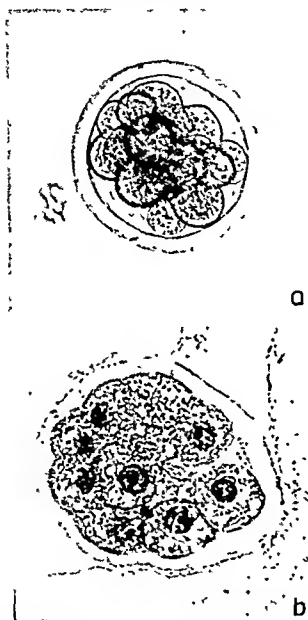


FIG. 10. Morulae. (a): Living sheep morula. Magnification: $\times 200$. (b): Section of a cat morula, showing the tendency for the smaller cells to gather near one pole and the larger near the other. Magnification: $\times 330$. (By courtesy of E. C. Amoroso.)

equal in size and, in the embryos of the ewe, goat, sow, and cat, the smaller cells are gathered mostly toward one pole and the larger cells toward the other.

B. Early Differentiation of Cell Types

The next step is the formation, within the mass of cells, of a small fluid-filled cavity, which is the beginning of the *blastocoele*; the embryo

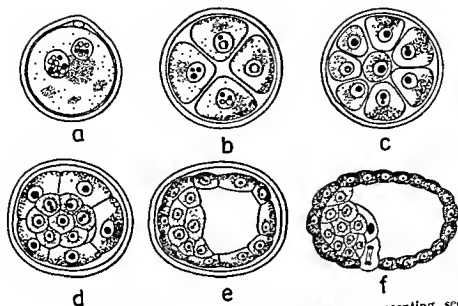


FIG. 11. Blastocyst formation in the rat. Diagrams representing sections of embryos stained for mucopolysaccharides. According to Daley (24), a coarser granulation becomes evident in parts of the 4-cell embryo (b); in the 8-cell embryo (c), this feature is said to distinguish those blastomeres that will form the trophoblast. (d): Early blastocyst with first signs of the blastocoele and the segregation of embryonic and extraembryonic cells. (e): Blastocyst with enlarging blastocoele and distinct inner cell mass. (f): Cells are believed to grow in from the trophoblast to form the endoderm. Redrawn and slightly modified from original drawings of Daley (24).

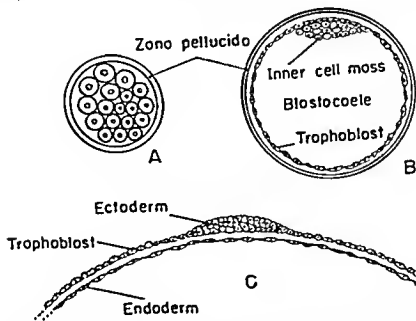


FIG. 12. Blastocyst formation in the sheep. Diagrams representing sections of sheep embryos. (A) Morula 5 days after coitus. (B) and (C): Blastocysts at 8 and 12 days, respectively, after coitus. Drawn from data of Boyd and Hamilton (17).

is now termed an early *blastocyst* (Figs. 11, 12, 13, and 14). The cavity soon enlarges—in the rodents this happens with little or no change in the total volume of the embryo, but in the domestic animals the total volume increases considerably. In the latter group, the blastocyst becomes virtually a bag of fluid within 2 or 3 days. The structural changes in-

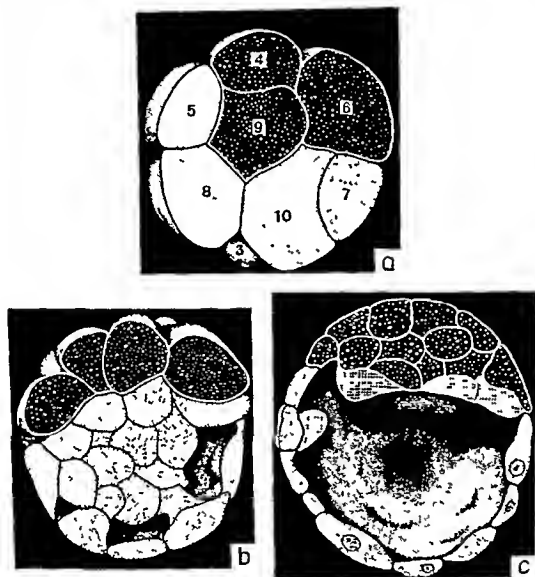


FIG 13 Blastocyst formation in the goat. Diagrams of sections of goat embryos to illustrate early differentiation of cell types, according to Amoroso *et al.* (2). (a). Morula 98 hours after coitus (b). Early blastocyst at 134 hours. (c) Blastocyst at 156 hours. White stipple on black = formative cells, stipple = trophoblast cells, crosshatch = endoderm. From Amoroso *et al.* (2)

involved in blastocyst growth are still incompletely understood and authorities differ in their interpretations. The processes may be said to be concerned, in general, with the separation of a group of "embryonic" or "formative" cells from which the fetus itself will develop, and of a group of "extraembryonic" cells which later form the fetal membranes, including the fetal placenta.

In rat embryos, according to Daleq (24), the formative cells, constituting the *inner cell mass*, are surrounded early by the extraembryonic cells, constituting the *trophoblast*. Later, a layer of cells comes to line the inner surfaces of trophoblast and inner cell mass and make up the *endoderm*; these cells are believed to have migrated from the trophoblast (see Fig. 11).

From the data presented by Boyd and Hamilton (17), the changes in the sheep seem to follow similar lines, except that the endoderm is thought to arise by migration or delamination of cells from the inner cell mass. In addition, the inner cell mass later becomes intercalated in the trophoblast layer and then projects above this layer as a *germinal disk*, consisting of *embryonic ectoderm* (see Fig. 12).

In the goat, Amoroso *et al.* (2) have described some distinctively large cells at one pole of the morula that evidently go to form the embryonic ectoderm while the trophoblast cells develop separately beneath this group. The endoderm is held to arise from trophoblast cells (see Fig. 13). Essentially the same process seems to occur in blastocyst development in the sow (32).

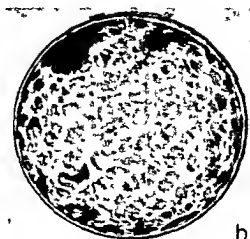
C. Cell Lineage

In some invertebrates it is possible to trace back certain of the organ-forming regions to early embryos, even to the undivided egg. Mammalian embryos, however, show evidence of differentiation relatively late, and the blastomeres of the early cleavage stages are each believed to be capable of forming all parts of the body. It has been demonstrated that the blastomeres of the 2-cell rat embryo can be separated experimentally and, on transfer to a suitable host, each can develop into an entire animal (44). Normal young rabbits have been born from 2-cell embryos in which one blastomere had been destroyed (51), and apparently normal blastocysts and implanted embryos have developed from 4-cell embryos in which three blastomeres had been destroyed (52). Nevertheless, there is evidence of some degree of determination in the early cleavage stages of mammalian embryos: Daleq and his associates have reported that by histochemical methods certain regions of the egg during fertilization and of the blastomeres of 2- and 4-cell embryos

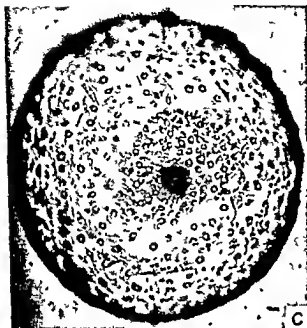
FIG. 14. Blastocysts. (a), (b) and (c): Living dog blastocysts at different stages of expansion (0-7 days after mating). In (a) and (b), the inner cell mass is seen in profile and, in (c), in plan view. Magnification: $\times 99$. (d): Living cow blastocyst (about 9 days). Magnification: $\times 225$. (e), (f), and (g): Sections of cat blastocysts (0-8 days), showing segregation of inner cell mass, differentiation of endoderm cells, and intercalation of embryonic ectoderm with trophoblast. Magnifications: $\times 405$, 450 , and 252 , respectively. (By courtesy of E. C. Amoroso.)



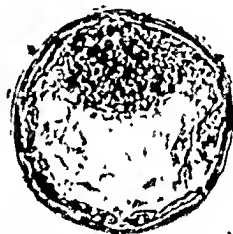
a



b



c



d



e



f

g

can be shown to contain material that will later characterize either the trophoblast or the inner cell mass (24). Figure 11 shows how, in sections of rat eggs and embryos stained for mucopolysaccharides, a coarser granulation becomes evident in parts of two blastomeres of the 4-cell stage. This seems to identify the regions of cytoplasm that will later form part of the trophoblast. Early recognition of cell types is also reported by Amoroso *et al.* (2) (see Fig. 13).

D. Rates of Cleavage and Volume Changes

The rate at which cleavage occurs has been found to vary in a rather unpredictable way among different animals. Here again, the data for domestic animals (Fig. 15) lack precision because they are so often

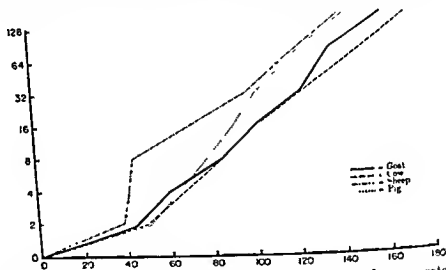


FIG. 15. Rates of cleavage. Graph summarizing data on cleavage rates in some domestic animals. Ordinate: number of cells composing embryo. Abseissa: time in hours after coitus. From Amoroso *et al.* (2).

related to the time of coitus rather than to the time of ovulation. In general, the embryos of domestic animals and laboratory rodents cleave at a slower rate than do those of the rabbit. The rabbit embryo reaches the 16-cell stage in just under 2 days from coitus, while rat and mouse embryos take 3 to 4 days and embryos of the larger domestic animals take 3 to 5 days. The blastocyst stage in the ewe, goat, and sow is reached in about 6 days and in the cow in about 8 to 9 days.

It has already been noted that the total mass of cytoplasm actually decreases during cleavage. Some measurements are recorded by Hamilton and Laing (30). From these it appears that the total volume of the cytoplasm in the 1-cell cow egg is about 900,000 cu. μ , compared with a volume of the order of 700,000 cu. μ in the 8-cell embryo: this

represents a diminution in cytoplasmic volume of about 20%. Corresponding figures for the sheep were about 1,000,000 and 600,000 $\text{cu } \mu$ a loss of about 40%. In the ferret and mouse there were found to be losses of about 30 and 25%, respectively. The reduction in cytoplasmic volume presumably can be ascribed to the utilization of food stores (the yolk or deutoplasm), which initially would take up appreciable space within the cytoplasm. This catabolism releases the energy required for cleavage.

E Parthenogenesis

Some animals, such as certain insects, reproduce only parthenogenetically, there is no fertilization, and development begins spontaneously. Among other animals, such as sea urchins and newts, reproduction is normally sexual but parthenogenetic development can be induced experimentally with a variety of agents (see Section IV E). Sometimes the parthenogenetic embryos grow into functional adults, particularly if "regulation to diploidy" has occurred (54). Commonly, this involves failure of abstriction of the second polar body, as a result, two chromosome groups are formed in the egg and thus the diploid state is established. Haploid parthenogones seldom develop far in vertebrates.

In mammals, mechanisms by which either haploid or diploid parthenogenesis could take place are well recognized, and there are several reports of induced early parthenogenetic development in the eggs of laboratory animals (11). So far, however, it is only in the rabbit that induced parthenogenesis has been claimed to have been followed by the birth of viable young (45, 46, 47). Though many investigators have described the apparent spontaneous parthenogenetic development of ovarian and tubal eggs in mammals, it is likely that all or the great majority of these eggs were in fact undergoing no more than a degenerative fragmentation. Nevertheless, the possibility does remain that very occasionally a parthenogenetic mammal arises spontaneously and survives to maturity. Such individuals would be difficult to detect, since successful development would probably have been contingent upon regulation to the diploid state. By employing various tests, including the identification of blood groups and the transfer of skin grafts, however, Balfour-Lynn (10) believes that he may have identified a human parthenogone.

VI MAINTENANCE OF THE EARLY EMBRYO

After fertilization, the embryo passes along the Fallopian tube and, in a few days, enters the uterus—this generally occurs when it is a morula or early blastocyst. Eventually, the embryo undergoes implantation in

the uterus, a process involving the formation of a placenta, to which both embryonic and maternal tissues contribute. The nutritive requirements of the embryo are met initially by the yolk material that it carries and perhaps by substances in the secretions of the Fallopian tube, and later by the products of the uterine mucosa. Ultimately, the embryo is maintained principally through the medium of the placenta.

A. *Transport of the Embryo*

It is likely that the early embryos, suspended in the tubal fluids, are transported by ciliary currents and by contractions of the Fallopian tube. Passage through the wider portion of the tube, the ampulla, is more rapid than through the narrower portion, the isthmus.

The rate of passage varies widely. In the opossum and wallaby, transport is so rapid that eggs still in the pronucleate stage of fertilization arrive in the uterus. In most mammals, however, the journey takes 3 to 5 days, irrespective of the size of the animal and the length of the Fallopian tube. Transport is relatively long in the dog (6-8 days) and cat (5-7 days).

Implantation in the uterus does not take place immediately upon arrival of the embryo; for a period, the blastocyst lies free within the uterine lumen. The interval is normally about 3 days in rodents; for domestic animals, only approximate estimates can be given: mare, about 7 weeks; cow, 20-30 days; ewe, 11-14 days; sow, 4-7 days; bitch and cat, about a week.

While the blastocyst is free in the uterus, it is moved about, presumably by contractions of the uterine walls. This may result in the transference of an embryo to the opposing uterine horn, a shift that is known as *internal migration*, and that has been reported to occur in all the domestic animals. In addition, in animals that bear several young in a given pregnancy, the embryos become arranged in such a way that their orientation within the lumen is always the same and their sites of implantation are more or less evenly distributed.

B. *Physiology of the Early Embryo*

Very many studies have been made on the metabolism of invertebrate eggs and early embryos, and much detailed information has been obtained. This has been possible principally because invertebrate eggs can be obtained readily in vast numbers and, even in the laboratory, conditions can easily be achieved in which fertilization and development will proceed in a normal manner. Investigations on mammalian eggs, on the other hand, have had to be made on much smaller numbers and are

further hampered by the fact that proper media for fertilization and cleavage outside the body have still to be perfected. Nevertheless, encouraging progress has been made.

It is known that mammalian eggs and early embryos can tolerate short periods of exposure to experimental conditions outside the body. Transfer from one animal to another has been effected in several different species (including cows, ewes, goats, and sows) with the subsequent birth of normal young as the outcome (64). Two-cell sheep embryos have even been transferred to rabbits and there have developed normally to the blastocyst stage (9). Chang (21) has shown that unfertilized rabbit eggs can survive storage *in vitro* at 10°C. for 48 to 72 hours and at 0°C. for 24 to 48 hours, and yet undergo normal fertilization when transferred to suitable hosts. Rabbit eggs during fertilization have even been found to withstand freezing to -190°C. and still be able subsequently to pass through a few cleavage divisions in culture (53).

Some success has been achieved in the *in vitro* culture of mammalian embryos. Rabbit embryos have developed from early cleavage to well-grown blastocysts in media containing serum (36, 48). Though other constituents of the medium could be varied without detriment, omission of the serum precluded the occurrence of more than a very few cleavage divisions. Mouse embryos, hitherto rather refractory material, have now been cultured from the 2-cell stage to the blastocyst in media made up from crystalline serum albumin, glucose, lactate, and a buffer mixture (63).

Measurements have been made of the oxygen consumption of rat and rabbit embryos, and these suggest that a single embryo utilizes about 0.5 to 1.0 m μ l. O₂/hr. (1 m μ l. = 10⁻⁶ ml.) (28, 43). There is evidence that the cytochrome oxidase system is present in these early tissues.

Biochemical studies have been made on the fluid contents of rabbit blastocysts just before and during implantation (days 6 and 7 from coitus), and also on the following day, when implantation is further advanced. The fluid was found to contain very little protein or glucose on day 6, more on day 7, and amounts approaching the serum concentration on day 8. Data showed that these substances had passed to the blastocyst from the maternal blood stream. Concurrently, the phosphorus content doubled and the chlorides increased about threefold. The concentrations of potassium and especially bicarbonate, however, were distinctly higher on day 6 than on day 7, and fell to maternal serum levels as implantation proceeded. Water-soluble vitamins—thiamin, riboflavin, and especially nicotinic acid and vitamin B₁₂—were also present in assayable amounts in the blastocyst fluid (20, 33, 31, 39).

Clearly, the biochemical architecture of the embryo, at least during the later cleavage stages, has its own peculiarities, which presumably betoken special features of embryonic metabolism. Their full significance remains to be elucidated, but the indications are that the mammalian embryo has some measure of nutritional independence, in spite of its small stores of yolk material. At least during cleavage, the demands that the embryo makes upon its environment may be principally of a physical nature—water, a supply of oxygen at a particular partial pressure, and a suspending medium that is osmotically suitable in respect of both ionic constituents and colloids. Later, when growth begins, an outside source of food material is clearly necessary; this must be supplied by substances in the free fluids of the genital tract and by materials absorbed through the placenta. The former are of particular importance in those animals in which the unattached blastocyst remains for a long while in the uterine lumen.

C. The Tubal and Uterine Environments

The Fallopian tubes are lined with nonciliated, as well as ciliated, columnar cells, the former having a secretory function. The rate of secretion in the tube is highest during estrus, when it amounts, in the rabbit, to about 0.79 ml. in 24 hours (12). Identified constituents of the secretion include mucoprotein, glycogen, phospholipid, and lactate. Glucose and fructose are not present in significant amounts. The oxygen partial pressure was found to be about 45 mm. Hg in the estrous rabbit; this is approximately in equilibrium with the arteriolar supply and is sufficient to maintain an essentially aerobic environment (13, 14, 29).

The uterine fluid in early pregnancy is of a distinctive character and is clearly suited to the nutrition of the embryo. This feature has long been recognized; indeed, the fluid was first termed *uterine milk* nearly 300 years ago. The material is derived from the secretions of cells in the mucous membrane and uterine glands, and contains much cellular debris, leucocytes, and some red cells. It is especially copious in animals with epitheliochorial and syndesmochorial placentae, namely, the mare, cow, ewe, and sow. In these animals, uterine milk probably contributes a large proportion of nutrients to the embryo throughout gestation. Its chemical composition varies a little between species, the protein content being higher in the mare (18%) than in the ruminants (10–11%), and the fat content much lower in the mare (0.006%) than in the ruminants (about 1%). It does not contain detectable amounts of glucose, fructose, or glycogen. The cellular debris derived from broken-down mucosal tissue is also of nutritive value; for this purpose, dissolution is unnecessary, as the trophoblast cells are known to be actively phagocytic (1).

REFERENCES

- 1 Amoroso, E C, in "Marshall's Physiology of Reproduction" (A S Parkes, ed), Vol 2, 3rd ed, Chapt 15 Longmans, Green, London, 1952
- 2 Amoroso, E C, Griffiths, W F B, and Hamilton, W J, *J Anat* 76, 377 (1942)
- 3 Austin, C R, and Bishop, M W H, in 'The Beginnings of Embryonic Development' (A Tyler, R C von Borstel, and C B Metz, eds), p 71 American Association Advancement Science, Washington, D C, 1957
- 4 Austin, C R, and Bishop, M W H, *Biol Revs Cambridge Phil Soc* 32, 296 (1957)
- 5 Austin, C R, and Bishop, M W H, *Proc Roy Soc B* 149, 241 (1958)
- 6 Austin, C R, and Braden, A W H, *Australian J Biol Sci* 6, 674 (1953)
- 7 Austin, C R, and Braden, A W H, *Australian J Biol Sci* 7, 543 (1954)
- 8 Austin, C R, and Braden, A W H, *J Exptl Biol* 33, 358 (1956)
- 9 Averill, R L W, *Proc 3rd Intern Congr Animal Reproduction Cambridge, Sect III*, p 7 (1956)
- 10 Balfour-Lynn, S, *Lancet* 1, 1071 (1956)
- 11 Betty, R A, 'Parthenogenesis and Polyploidy in Mammalian Development' Cambridge Univ Press, London and New York, 1957
- 12 Bishop, D W, *Am J Physiol* 187, 347 (1956)
- 13 Bishop, D W, *Anat Record* 125, 631 (1956)
- 14 Bishop, D W, *Proc 3rd Intern Congr Animal Reproduction Cambridge, Section I*, p 53 (1956)
- 15 Bishop, D W, and Tyler, A, *J Exptl Zool* 132, 575 (1956)
- 16 Bishop, M W H, and Austin, C R, *Endeavour* 16, 137 (1957)
- 17 Boyd, J D, and Hamilton, W J, in "Marshall's Physiology of Reproduction" (A S Parkes, ed), Vol 2, 3rd ed, Chapt 14 Longmans, Green, London (1952)
- 18 Braden, A W H, Austin, C R, and David, H A, *Australian J Biol Sci* 7, 391 (1954)
- 19 Brambell, F W R, in "Marshall's Physiology of Reproduction" (A S Parkes, ed), Vol 1, 3rd ed, Chapt 5 Longmans, Green, London, 1956
- 20 Brambell, F W R, and Hemmings, W A, *J Physiol (London)* 108, 177 (1949)
- 21 Chang, M C, *J Exptl Zool* 121, 351 (1952)
- 22 Chang, M C, in 'The Beginnings of Embryonic Development' (A Tyler, R C von Borstel, and C B Metz, eds), p 169 American Association Advancement Science, Washington, D C, 1957
- 23 Chang, M C, and Pincus, C, *Physiol Revs* 31, 1 (1951)
- 24 Daleq A M, *Proc Soc Study Fertility* 7, 113 (1955)
- 25 Dawson, A B, and Friedgood H B, *Anat Record* 76, 411 (1940)
- 26 De Robertis, E D P, Nowinski, W W, and Saez, F A, 'General Cytology,' 2nd ed Saunders, Philadelphia Pennsylvania, 1954
- 27 Frank, A H, *U S Dept Agr Circ No* 567 (1952)
- 28 Gridhandler, L, Hafez, E S E, and Pincus, C, *Proc 3rd Intern Congr Animal Reproduction Cambridge Section I*, p 48 (1956)
- 29 Hadck, R, *Anat Record* 121, 187 (1955)
- 30 Hamilton, W J, and Laing J A, *J Anat* 80, 191 (1916)
- 31 Hartman, C C, in "Sex and Internal Secretions" (E Allen, ed), 2nd ed, p 630 Bailliere, Tindall, and Cox, London, 1939

32. Heuser, C. H., and Streeter, G. L., *Carnegie Inst. Contribs. Embryol.* No. 109, 20, 1 (1929).
33. Jacobson, W., and Lutwak-Mann, C., *J. Endocrinol.* 14, xix (1956).
34. Kodicek, E., and Lutwak-Mann, C., *J. Endocrinol.* 15, liii (1957).
35. Laing, J. A., in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 3, Chapt. 17. Butterworths, London, 1957.
36. Lewis, W. H., and Gregory, P. W., *Science* 69, 226 (1929).
37. Liche, H., *Nature* 143, 900 (1939).
38. Lillie, F. R., *Science* 38, 524 (1913).
39. Lutwak-Mann, C., *J. Embryol. Exptl. Morphol.* 2, 1 (1954).
40. Mann, T., Polge, C., and Rowson, L. E. A., *J. Endocrinol.* 13, 133 (1956).
41. Millar, R., *Australian Vet. J.* 28, 127 (1952).
42. Mitchison, J. M., *Symposia Soc. Exptl. Biol.* 6, 105 (1952).
43. Nicholas, J. S., *Quart. Rev. Biol.* 22, 179 (1947).
44. Nicholas, J. S., and Hall, B. V., *J. Exptl. Zool.* 90, 441 (1942).
45. Pincus, G., *Proc. Natl. Acad. Sci. U. S.* 25, 557 (1939).
46. Pincus, G., *J. Exptl. Zool.* 82, 85 (1939).
47. Pincus, G., and Shapiro, H., *Proc. Am. Phil. Soc.* 83, 631 (1940).
48. Pincus, G., and Werthessen, N. T., *J. Exptl. Zool.* 78, 1 (1938).
49. Rothschild, Lord, "Fertilization." Methuen, London, 1956.
50. Rowson, L. E. A., *Brit. Vet. J.* 3, 334 (1955).
51. Seidel, F., *Naturwissenschaften* 15, 355 (1952).
52. Seidel, F., *Naturwissenschaften* 13, 306 (1956).
53. Smith, A. U., in "Mammalian Germ Cells" (C. E. W. Wolstenholm, ed.), p. 217. Churchill, London, 1953.
54. Tyler, A., *Biol. Revs. Cambridge Phil. Soc.* 16, 291 (1941).
55. Tyler, A., *Physiol. Revs.* 28, 160 (1946).
56. Van Demark, N. L., and Hays, R. L., *J. Animal Sci.* 10, 1063 (1951).
57. Van Demark, N. L., and Hays, R. L., *Am. J. Physiol.* 170, 516 (1952).
58. Van Demark, N. L., and Hays, R. L., *Iowa State Coll. J. Sci.* 28, 107 (1953).
59. Van Demark, N. L., and Moeller, A. N., *Am. J. Physiol.* 165, 674 (1951).
60. Van der Stricht, R., *Arch. Biol. Liège* 25, 365 (1911).
61. Walton, A., and Austin, C. R., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), Vol. 1, Part 2, 3rd ed., Chapt. 10. Longmans, Green, London, 1959.
62. Warbritton, V., McKenzie, F. F., Berliner, V. R., and Andrews, F. N., *Proc. Am. Soc. Animal Production* 30, 142 (1937).
63. Whitten, W. K., *Nature* 179, 1081 (1957).
64. Willett, E. L., *Iowa State Coll. J. Sci.* 28, 83 (1953).
65. Wotton, R. M., and Village, P. A., *Anat. Record* 110, 121 (1951).

CHAPTER 13

Implantation, Development of the Fetus, and Fetal Membranes

ELMER B. HARVEY

	<i>Page</i>
I. Introduction	433
II. Implantation	436
A. Artiodactyla	436
B. Perissodactyla	437
C. Carnivora	437
III. Yolk Sac and Vitellochorion	438
A. Artiodactyla	440
B. Perissodactyla	441
C. Carnivora	441
IV. Amnion	442
A. Artiodactyla	444
B. Perissodactyla	444
C. Carnivora	445
V. Chorion, Allantois, Allantochorion	445
A. Artiodactyla	446
B. Perissodactyla	450
C. Carnivora	450
VI. Chorioallantoic Placenta	451
A. Epitheliochorial Placenta	453
B. Syndesmochorial Placenta	456
C. Endotheliochorial Placenta	459
VII. Aging and Fetal Development	461
References	466

I. INTRODUCTION

For the continued existence of the normal zygote, embryo, and fetus, it is necessary that their structure be so designed for each developmental period that an adequate physiological performance is ensured. Because of the changes (differentiation and growth) that occur in the developing embryo throughout the gestation period, the requirements for existence progressively change. These needs are met by development of new or expanded embryonic or extraembryonic structures. The morphology and progressive development of structures which make possible a complex intrauterine existence of embryos and fetuses in domestic animals are the main topic of this chapter.

The animal groups dealt with in this book conveniently fall into three orders: Artiodactyla (split-toed): pigs, cattle, sheep, goats, and deerlike animals; Perissodactyla (single-hoofed or uneven-toed): horses,

elephants, camels, hippopotami, etc.; and Carnivora (meat-eaters): dogs, and doglike animals (foxes, wolves, etc.), and cats as well as catlike animals (mountain lion, tigers, etc.). Our attention will be focused on the domestic animals; nonetheless, studies of details in these species should lead toward a broad understanding of implantation, fetal membranes, and fetal development because characteristics do not differ, except in detail, between members of the species within any one order.

In the broad sense, a placenta is any intimate apposition or fusion of fetal organs to the maternal tissues for physiological exchange (64). Membranes to which this definition may apply either in part or in total are the trophoblast, yolk sac, amnion, chorion, allantois, allantoamnion, and allantochorion.

Commonly, the chorioallantoic placenta is referred to as "the" placenta. This placenta takes on various forms in different species, but is relatively constant in any order. Classification may be based upon gross shape or, preferably, upon its finer structure and relationship with maternal tissue (Table I).

Implantation occurs when the embryo becomes fixed in position through a reaction involving maternal and embryonic or extraembryonic tissue. In some species, such as the martin and long-tailed weasel, the blastocyst may be dormant in the uterus for weeks (delayed implantation) before becoming fixed in position (89). Implantation in artiodactyls and perissodactyls occurs after formation of the definitive extraembryonic membranes; however, in other orders, i.e., Carnivora, Lagomorpha, Rodentia, Primates, this may not be the case (64).

In mammals with more than one young per litter, blastocysts are spaced evenly throughout the uterine horns and implantation occurs first at the oviducal end of the uterus. When twins are present in bicornuate uteri of ruminants, usually one embryo is found in each horn and the sites of implantations in the two horns are symmetrically placed near the middle. In eutherian mammals, orientation of the germ disk may be toward the broad ligament (mesometrial) or away from the broad ligament (antimesometrial). Attachment with the germ disk in one of these directions is relatively constant within any order, but depth of implantation may vary. In domestic animals, the germ disk orients antimesometrially and implantation is circumferential or diffuse [necessarily superficial (64)].

Nutritive materials ("embryotrophe") for the embryo and fetus may be derived either from maternal circulation ("hemotrophe") or through secretory or degenerative products of the endometrium ("histotrophe," 40).

TABLE I
TISSUES SEPARATING MATERNAL AND FETAL BLOOD^a

	Maternal tissue			Fetal tissue			Gross form	Example
	Endo-thelium	Connective tissue	Epi-thelium	Uterine lumen	Tropho-blast	Connective tissue	Endo-thelium	
chorio-vascular	+	+	+	+	+	+	+	Pig, horse
chorio-epithelial	+	+	-	+	+	+	+	Ruminants
chorio-endothelial	+	- (+) ^b	-	-	+	+	+	Carnivora
chorio-vascular	-	-	-	-	-	-	+	Insectivora, Chiroptera, Primates, Castoridae, Aplodontiidae, Sciuridae
chorio-epithelial	-	-	-	-	-	+	+	Geomyidae, Ochotonidae
chorio-endothelial	-	-	-	-	-	-	+	

^aClassification of placental types modified from Grosser (40) and Mossman (64), with the addition of a type combining hemochorial and endothelial characters. The designation of examples is largely based on Mossman, but revised, and hemochorial with hemoendothelial areas has been added by this writer. (By permission of H. W. Mossman and Carnegie Institution, Washington, D. C.)

^bCollagen and reticular fibers (of unknown origin) surround vessels. These may constitute an effective barrier.

The intimacy of the fetal and maternal blood in the chorioallantoic placenta determines the means by which an embryo obtains its nutriment. In those fetuses that establish an epitheliochorial and syndesmochorial placenta (Table I), histotrophe furnishes a considerable supply of nutrient throughout gestation. In Carnivora, which develop an endotheliochorial placenta, histotrophe is of considerable importance early in gestation. Although histotrophe may serve as a nutrient source early in these species, its apparent usefulness is reduced after establishment of the chorioallantoic placenta in its final form.

II. IMPLANTATION

Since embryology in the sow has been studied more extensively than in other domestic animals, conditions in this species will be described first in the discussion to follow.

A. *Artiodactyla*

Passage of the pig blastocyst through the oviduct takes about $3\frac{1}{2}$ days (Chapter 12). It remains free in the uterine horn for about 6 or 7 days (9–10 days postcoitus). During this period of uterine existence, blastocysts become spaced in the horn of the uterus and become oriented so that the germ disk faces toward the mesometrial quadrant of the uterus (45). Orientation of the blastocyst occurs in a predictable way in most of the eutherian mammals, but its mechanism remains one of the more puzzling problems of mammalian embryology. Markee (61) believes that the major factor involved in spacing the embryos is the peristaltic contraction of the uterine muscle. He further postulates a recoil action against normal peristalsis movement which allows transfer of ova into the other uterine horn.

Attachment of blastocysts in the sow, as well as in other domestic animals, is of an extremely loose nature, and some controversy centers around the actual time and method of first attachment. Green and Winters (37) report that blastocysts of sheep are attached to the uterus by means of an adhesive, mucinlike material at 10 days postcoitus. Wimsatt (81) disagrees with these workers. Though assumed in many species, a similar type of implantation has not been demonstrated in other domestic animals.

Winters *et al.* (82) and Green and Winters (36, 37) report that a loose attachment of the blastocyst occurs in the cow, ewe, and sow at about the 11th day. Davies (20), on the other hand, insists that first attachment occurs at about the 22nd day, when there is erosion of mater-

nal tissue in the presence of the allantochorionic membrane. Melton *et al.* (63) and Chang (13) conclude that first attachment of the bovine embryo does not take place till the end of the first month; they describe it as a gradual process. Hammond (43), however, believes that some reaction between the embryo and endometrium is necessary in order to maintain the corpus luteum of pregnancy and that this reaction must take place before the normal time of atrophy of the cyclic corpus luteum (Chapter 7). This would suggest some form of attachment earlier than 22 days and 30 days, as reported by Davies and Melton for the ewe and cow, respectively.

B. *Perissodactyla*

Implantation does not occur in the mare for a period of about 8 weeks, at which time chorionic villi begin to penetrate into uterine sulci (31). Prior to this time, and in early periods of attachment, the chorion is held in apposition to the uterine mucosa by pressure of fetal fluids (90). By the 10th week, chorionic villi penetrate sulci and by the 14th week [15.5 cm. crown-rump length (C-R)], complete attachment is attained.

C. *Carnivora*

Blastocysts of carnivores become spaced equally throughout the lengths of the uterine horns, as in the sow. As a rule, the embryos lie antimesometrially with the embryonic axis almost transverse to the long axis of the uterine horn. In the cat, at about 13 days, implantation sites are visible as uterine swellings (locules: 9-10 mm. diameter), while in bitches these are visible at about 15 days postcoitus (2). In *Carnivora* implantation is of a central (superficial) type, as in the other domestic animals. The blastocyst, attaining a large size, rests in the uterine cavity and comes into contact with uterine epithelium over most of its circumference. Before chorioamniotic folds form, attachment occurs through invasion of the uterine mucosa by villi from the vitellochorion (Fig. 1) (44).

By the end of the implantation period two different placental regions may be recognized in *Carnivora*: (a) choriovitelline placenta, which is applied to the mesometrial wall of the implantation chamber; and (b) the chorionic, constituting the antimesometrial wall of the locular enlargement (Fig. 1). Both of these regions are involved later in formation of the chorioallantoic placenta (Fig. 2). This transformation is brought about when vascular allantoic mesoderm reaches and invades mesoderm of the chorion and vitellochorion (24-26, 38).

III. YOLK SAC AND VITELLOCHORION

Considerable confusion exists in the literature concerning the term yolk sac. Mossman (64) prefers to use the terms unilaminar yolk sac to designate the trophoblastic sac enclosing the blastocoele, and bilaminar yolk sac when endoderm lines the inner aspect of the trophoblast. In cases where mesoderm insinuates between these two layers it is designated as trilaminar. We restrict the term yolk sac for the endodermal membrane enclosing the archenteron. The vitellochorion is the bilaminar membrane formed when yolk-sac endoderm is fused with or closely apposed to trophoblast. When mesoderm grows between these two it can then be designated as the trilaminar vitellochorion. The choriovitelline placenta would be then composed of the vitellochorion membrane, fused or closely apposed to uterine endometrium for purposes of physiological exchange (Fig. 1).

If one adheres to strict definitions, the yolk sac of the sow, ewe, and

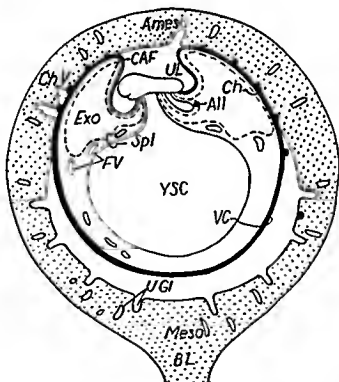


FIG. 1. Diagram of cross section of the Carnivora uterine locule at an early implantation stage. Chorioamniotic folds (CAF) have not met. The vitellochorion (VC) is being converted to a trilaminar vascular structure by invasion of mesoderm. Note central (superficial) implantation with the embryo dorsum in the anti-mesometrial (Ameso) quadrant. By permission of H. W. Mossman (64) and the Carnegie Institution of Washington, Washington, D. C.

KEY TO LETTERING ON FIGS. 1-8

AC	Allantochorionic membrane	I Cot	Intercotyledonary Area
All	Allantois	LZ	Labyrinthine Zone
All Am	Allantoamnion	M	Muscularis
All V	Allantoic Vesicle	MA	Maternal Artery
Am	Amnion	Meso	Mesometrial
Am C	Amniotic Cavity	MS	Maternal Septa
Am Ch	Amniochorion	MV	Maternal Vein
Ameso	Antimesometrial	NT	Necrotic Tip
Am P	Amniotic Pustule	Ped	Pedicle
BL	Broad Ligament	PV	Primary Villous
CA	Chorioallantois	Smpl	Somatopleure
CAF	Chorioamniotic Fold	Spl	Splanchnopleure
Car	Caruncle	St	Stroma
Ch	Chorion	Synt	Syncytiotrophoblast
Ch V	Chorionic Villi	UE	Uterine Epithelium
Cot	Cotyledon	U Gl	Uterine Gland
Cyt	Cytotrophoblast	UL	Uterine Lumen
Exo	Exocoelom	Um C	Umbilical Cord
FA	Fetal Artery	V	Villous
FV	Fetal Vessel	VC	Vitellochorion
GLZ	Glandular Zone	YSC	Yolk Sac Cavity
Hem	Hematoma		

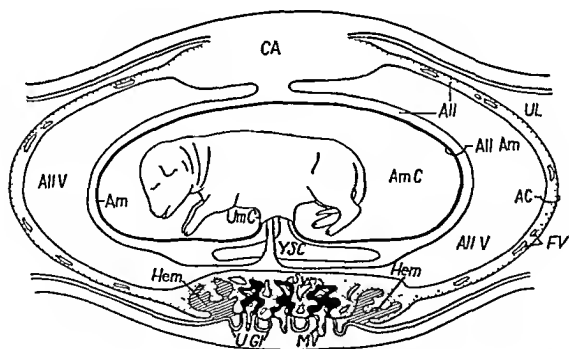


FIG. 2. Longitudinal section of Carnivora (bitch or cat) uterine locule with a late fetus, showing the hematoma (Hem) and chorioallantoic placenta (CA) in diagrammatic detail. See also Fig. 8c. For key to lettering see legend to Fig. 1. By permission of H. W. Mossman (64) and the Carnegie Institution of Washington, Washington, D. C.

cow cannot be considered a placenta at any time in the development of the embryo. It does not become closely apposed or fused with maternal tissue as it does in the dog, cat, mare, and other species (64-65). This structure, however, does play a part in the nutrition of the early developmental stages in that "uterine milk" passes through this membrane, at least passively if not actively.

A. Artiodactyla

Along with the precocious expansion of the trophoblastic vesicle in the sow, the endoderm lengthens and forms a tubular yolk sac extending throughout the chorion and lightly fused to it. Blood vessels appear

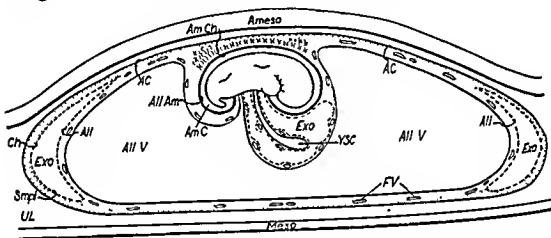


FIG. 3. Longitudinal section of sow uterine locule with an early embryo. Note fusion of amniochorion (Am Ch) and the regressing but vascular yolk sac. For key to lettering see legend to Fig. 1. By permission of H. W. Mossman (64) and the Carnegie Institution of Washington, Washington, D. C.

early in the yolk sac and form a complete capillary mesh. A vascular yolk sac is thus in contact with the chorion, even though the area of fusion is very limited and soon to be relinquished. This constitutes a (yolk sac) placenta that remains efficient until its function is transferred to the more highly developed chorioallantois (45). Hemopoiesis may be observed in yolk sacs of pig embryos from 5 to 15 mm. C-R and is at its height at about the 10-mm. stage (51). Jordan (51) described filaments (villi) in the yolk sac. Though not present in the gut endoderm, Heuser (45) suggests that these villi may elaborate enzymes for further digestion of "uterine milk" (histotrophe). In the latter part of gestation the yolk sac, displaced by the developing allantois and shrinking to a thin membrane, finally disappears before parturition (Fig. 3).

In the ewe and cow, the yolk sac is large in early stages but is rapidly

outgrown by the allantois. By the 13th day in the ewe, one-third of the yolk-sac circumference is surrounded by extraembryonic coelom. At the 17th day, separation of the yolk sac from the chorion is complete; it is gradually pushed to one side by expansion of exocoelom, and by the 25th day is reduced to a solid rod of cells with a few blood vessels on its surface. It finally disappears before term (90).

A vitelline circulation is initiated before the yolk sac severs its connection with the chorion. Thus a choriovitelline placenta is established which may be functional till about the 17th day of pregnancy.

B. *Perissodactyla*

Splitting of extraembryonic mesoderm is never complete in the mare embryo. Thus, the yolk sac remains attached to the chorion in the antiembryonic region and never becomes an independent structure, as in the pig, cow, and sheep. Moreover, mesoderm does not completely invade the lower polar regions of the blastocyst until late in pregnancy. For a time, therefore, bilaminar vitellochorion is in apposition with uterine mucosa. In late stages, the yolk-sac connection is a slender one at the abembryonic pole and is of doubtful placental function (31).

The trophoblastic wall of the chorionic vesicle at the end of the 3rd week in the mare differs from that of the pig, cow, and sheep. Within the confines of the sinus terminalis it has columnar rather than cuboidal epithelium as in the artiodactyls. Outside the sinus are trophoblastic disks composed of groups of columnar cells which may help in attachment. Around the disks and circling the sinus terminalis are columnar cells with saclike extensions. They probably lie opposite gland mouth openings and may be involved in taking up more solid particles of "uterine milk" (histotrophe) (31).

At the 5th week a band of the trophoblast, approximately 7 mm. wide and surrounding the equator of the chorionic sac, thickens. This ring adheres to the uterine wall and probably strengthens the vitellochorionic attachment.

At the end of the 6th week the allantois begins to fuse with the chorion and to extend into the tissue of the vitellochorionic membrane. Thus, the yolk-sac membrane attachment to chorion diminishes and shifts from the equator to the poles of the chorionic sac. Hemopoiesis occurs in the yolk sac of the mare as in the pig.

C. *Carnivora*

In both the cat and dog, the yolk sac is completed while attachment of the blastocyst with the maternal tissue is being effected (24-26). The

yolk-sac cavity is at first very large; indentation of its upper wall by the embryo results in so-called "incomplete inversion" of the sac (65). The yolk sac is much reduced in later stages and becomes a separate and independent vesicle. Three stages in its development may be recognized. In the first stage, endoderm of the yolk sac is in contact with the trophoblast throughout the extraembryonic region and forms with the latter a bilaminar vitellochorion. The bilaminar vitellochorion is non-vascular and is completed in both species before implantation. In the second stage, the area of the bilaminar vitellochorion is replaced progressively by the trilaminar vitellochorion as extraembryonic mesoderm advances between the endoderm and trophoblast. Finally, in the third stage, this nonvascular trilaminar structure is invaded by vitelline vessels from the area vasculosa and is thus transformed into a vascular choriovitelline placenta. This placenta is established in the early somite stages and at this time becomes the principal placental organ (Fig. 1). Between the 21st and 24th day, when exocoelom extends into the area vasculosa and divides the vitellochorion into two layers, vascular splanchnopleure and nonvascular somatopleure, the choriovitelline placenta disappears. This process is not completed until some time after the vascular allantoic mesoderm has reached and made contact with chorion to initiate allantochorionic circulation (Section IV).

In its finer structure, the choriovitelline placenta consists of solid trophoblastic villi engaging the endometrium and penetrating the mucosa a variable distance. Invasion of these fetal villi by blood vessels remains scanty. Degenerating epithelium is absorbed, presumably by trophoblast for fetal nutrition (44). The mechanism of epithelial destruction underlying the vitellochorion needs investigation.

The yolk sac persists until term as a wrinkled, highly vascular structure which is attached by its extremities to the chorionic membrane. It has a hemopoietic function as well as a possible role in nutrition (Fig. 2).

IV. AMNION

Two distinct methods of amnion formation occur in eutherian mammals: by folding and by cavitation. The latter method is considered more specialized (64). In domestic animals, the amnion forms by folding.

Shortly after the primitive streak stage in these species, the embryonic disc appears to be depressed slightly into the blastocyst. As a result, somatopleure (chorionic ectoderm and somatic mesoderm) lies over the perimeter of the embryo. The somatopleure then grows over the embryo centripetally, whence the folds meet and fuse. Later, the outer layer

the mare (70) are the hippomanes. These are small, dark masses which may be present in the allantoic or amniotic fluids. In section, these are divisible into two areas: an outer, soft, lamellated cortex and an inner medullary part composed of granular material of cell debris, fat globules, and degenerating blood corpuscles. Schauder (70) suggests their derivation from endometrial cups. To date these have not been reported in carnivores.

The amniotic membrane does not serve as a placenta. However, the amniotic fluid undergoes changes in volume and changes in rate of water and sodium turnover. Also, it apparently stores detritus and maternal blood cells. Investigations are needed to establish its importance in fetal development and nutrition (10, 47).

A. *Artiodactyla*

The blastocyst of the sow has nearly reached its maximum length and is contained in a uterine locule before the chorioamniotic folds begin to enclose the developing embryo. In the sow embryo, the folds of somatopleure begin at the periphery of the embryonic disk just before the first somite is formed and fuse over the dorsal region of the embryo in about the 10-somite stage (40). A remnant of the chorioamniotic fusion, the amniotic umbilicus, is still present in the 5-mm. (39-somite) pig and is usually localized in the head region (64).

Chorioamniotic folds (somatopleure), which give rise to the amnion and chorion, begin about the 12th day in the ewe and become pronounced in the 13-day specimen, at which time the primitive streak has started to form. These folds are not complete by the 4-somite stage, but by the end of the 14th day chorion and amnion are separate. At this time the neural tube is closed back to the hind-gut region, and the allantois has formed and is growing laterally.

In the cow embryo, chorioamniotic folds begin in the 14th day and are completed sometime during the 18th day (82). If our interpretation of the descriptions by Winters *et al.* (82) is correct, the amnion forms at an earlier stage (presomite) in the cow than in the sow and ewe. By the 5-somite stage, unquestionably, the amnion is completed and the chorion separate from it.

B. *Perissodactyla*

Amnion formation in the mare embryo does not differ grossly from that of artiodactyls. Chorioamniotic folds begin about the 18th day (4-somite stage) and are fused at about the 21st day (20 somites). In its early stages (3-8 weeks), the amnion is tightly applied to the head fold and less so around the body of the embryo; at this time the amniotic

vesicle contains about 2 to 5 ml. of fluid. At terminal stages, the fluid volume increases 3 to 5 liters (42). Unlike the condition in artiodactyls, the allantois surrounds and fuses with the amnion to form a vascular allantoamnion.

Lining the inner border of the amnion are a number of protuberances ("amniotic pustules") rich in glycogen. These are formed by amniotic ectoderm about the 10th week. Their function is not known (31).

C. Carnivora

The mode of amnion formation in dogs and cats does not differ markedly from species of Artiodactyla and Perissodactyla. However, unlike these two orders, the amnion does not form amniotic pustules. The amnion remains free from the chorion after its initial separation and even though the amnion is surrounded by vascular allantois, these two membranes do not fuse as in the mare (44). Changes in amniotic fluid volume throughout gestation in the cat are discussed by Wislocki (85).

V. CHORION, ALLANTOIS, ALLANTOCHORION

In the literature one finds the term "chorion" applied to many structures, which are not homologous or even analogous. Mossman (64) argues effectively for a more restricted use of the term "chorion." He prefers to designate the outer fetal membrane, composed of trophoblast and somatic mesoderm (somatopleure) as chorion. When this layer becomes vascularized by the allantois, it is designated as allantochorion. If, as happens in some species, only a part of the chorion is vascularized by allantois, that part is designated as allantochorion, and the remaining portion should retain the term chorion. Mossman sees no reason for not using the term "chorion" loosely as long as the writer makes clear his meaning. We shall use the term chorion in its meaning as suggested by Mossman.

In some species the allantois grows out as a solid bud of tissue (primates, higher rodents), in which case this structure serves only to vascularize the chorion. In the domestic animals, however, this structure has a large central vesicle. In these, the allantois comes to occupy most of the exocoelom and fuses with amnion (allantoamnion). In the sow it fuses with the chorion and amnion to form an allantoamniochorion (Fig. 3).

Bremer (10) has shown that animals with a large allantoic vesicle have large functional Wolffian bodies (mesonephros) and a relatively thick placental membrane (allantochorion). This has been shown to be true in the pig, cat, and sheep, and appears to be the condition of other

species in the orders Artiodactyla, Perissodactyla, and Carnivora. More work, however, needs to be done to determine the universality of this rule (65). Gersh (33) questions Bremer's thesis; see also Wislocki and Bennet (86).

A. *Artiodactyla*

Separation of the amnion from the chorion completes formation of the definitive chorion. Further specialization of this structure occurs during and following fusion of allantoic membrane to the chorion.

The allantois in the 10-somite pig (time of fusion of amniochorion) is a small, crescent-shaped vesicle protruding from the hind-gut of the embryo. The points of the crescent are directed toward the anterior (cranial) end of the embryo. The allantois enlarges rapidly so that by the 39-somite (5 mm. C-R) stage it is considerably larger than the embryo and has an elaborate vascular pattern. The allantois lies in contact with the chorion for some time before actual fusion of the two layers takes place; at about the 7-mm. stage these two membranes begin to fuse together. By the time the embryo reaches 15 mm. C-R, fusion of the allantois to the chorion is completed. At this time the allantois is rounded at its ends and thus does not extend into the tips of the chorion (45). In later stages a constriction appears in the ends of the allantois which extend into the remaining exocoelom of the chorion. Even though the ends of chorions of adjacent embryos may overlies each other in the constricted region of the uterus (between the locules) they do not fuse together. Rather, they become atrophic following constriction of allantois in these tips (Fig. 3) (46).

In its early stages, the allantochoion is folded in accommodation to the rugae of the endometrium; later, villi from these folds grow into fossae or crypts in the uterine mucosa and branch. Mesenchyme and a rich vascular bed grow into these primary and secondary branches of the allantochoion. Wislocki and Dempsey (87) suggest that tumescence of these villi helps to anchor the placenta into position.

Areolae, pits, and vesicles begin to appear on the outer surface of the pig allantochoion at about the 12-mm. stage (30, 45, 80). The areolae are composed of a circular fold of allantochoion directed toward uterine epithelium and over gland mouths. Embedded in the mesenchyme and underlying the cuboidal cells lining the central disks of the areolae are rich venous plexi.

In the 15-mm. C-R stage, areolae are about 1 mm. in diameter and larger in diameter at mid-pregnancy. They may be either round or oval in shape, with complex folds at their base (45). Epithelium lining them is composed chiefly of simple columnar cells, with small densely staining

basophilic nuclei and pale-staining cytoplasm. The numerous vacuoles in these cells contain a substance having similar staining properties to "uterine milk" (45) and they also contain concentrations of glycogen, iron, and phosphatase (87).

The pits (irregular areolae), ranging from 1 to 15 times the diameter of the regular areolae, may be deep or shallow. Various number of folds and papillae project into their cavity. Vesicles (cystlike structures) that invaginate toward the allantoic cavity, and which may not be connected by a duct to the surface, also appear in the allantochorion of the sow. These vesicles have a more basophilic-staining epithelium than those which open to the surface and their function has not been investigated (45).

The sequence of events in the formation of the chorion, allantois, and allantochorion are similar within the ruminant members of the artiodactyls, but differ from those in the sow. The ewe has been studied most. Thus, it will serve as the representative of the ruminants in the discussion of these membranes. Any attempt to relate time, stage, and age development of fetuses with fetal membrane development is a nearly impossible task, since different workers have used different designations to indicate stages and ages of fetuses (see Section VI).

Separation of the amnion and chorion occurs between the 14th and 15th day in the ewe. At this time the allantois is a small bifid sac extending from the gut (37), but, according to Davies (20), the allantois does not appear until the 16th or 17th day, at which time the embryo has 7 somites. At the 14-somite stage the allantois is 7 mm. long. At 23 somites it is 12 mm. long and contains many areas of angioblastic activity. By the time the embryo is 5 mm. C-R, the allantois is 20 mm. long. In another embryo 5 mm. C-R (the twin of above), Davies indicates that the embryo has developed beyond its twin and that the allantois is 40 mm. long, richly vascular, and contains 3 ml. of fluid. In a 4 mm. C-R embryo developed still further than either twin, the allantois contains 7 ml. fluid and the allantois occupies one-half of the chorionic sac.

The allantois in sheep comes into apposition with the walls of the chorion by the 8-mm. stage and fusion is completed at the 10-mm. stage. Progressive fusion of the allantois in sheep occurs in a way similar to the pig (20). The chorion occupies both horns of the uterus at 14 days postcoitus (3). However, Green and Winters (37) report that the chorion only occupies three-fourths of one horn at 17 days.

Development of the allantois and its fusion with the chorion is similar in the cow and sheep. Winters *et al.* (82) states that the 22-day, 16-hour

bovine embryo and allantois are similar to the 16-day sheep embryo. According to Amoroso (2), the allantois fuses with the chorion at the 24th day in the pig, 22nd day in sheep, and 28th day in the cow.

By the 4th week (21-28 days) postcoitus, the allantochoion occupies both uterine horns of the ewe (Fig. 4). In the cow, 28 to 35 days pass before this event occurs.

Even though the allantois of the pig, cow, and ewe attains large dimensions, it does not surround the amnion as in the mare. Rather it extends as two arms from the ventral urachus and pushes the large amnion against the chorion. Thus the allantoic membrane is in contact

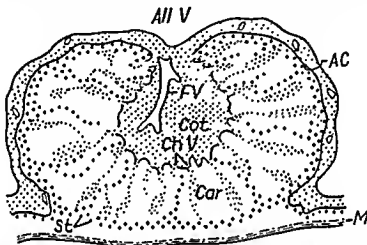


FIG. 5. Diagrammatic representation of a ewe placentome. Note that the caruncle is concave with the fetal cotyledonary tissue contained within its concavity. For key to lettering see legend to Fig. 1. By permission of H. W. Mossman (64) and the Carnegie Institution of Washington, Washington, D. C.

with the amnion (allantoamnion), chorion (allantochoion), and circumferentially surrounds the amniochorionic apposition or fusion (Fig. 4).

Necrosis occurs at the tips of the allantochoion in the ewe, as in the sow, but is less severe in the cow. According to Jenkinson (50), this necrosis is due to the restricted blood supply in these regions. Hammond (42) believes that fusion of adjacent allantochoion of twin fetuses is more likely in cows where necrosis is less severe than in the pig and ewe. Thus the likelihood of crossed circulation is increased in the cow. The higher incidence of freemartins in cattle than in sheep and pigs supports Hammond's contention.

Differentiation of the allantochoion in ruminants occurs when the blood supply underlying the trophoblast increases. The nature of the interaction between caruncles and fetal cotyledons has not been deter-

mined. The membranous chorion of the ruminants is avillous and is smooth over most of its surface except where it comes into contact with carunculae to form the cotyledons (Figs. 5, 6, and 7). Areolaelike structures form on the allantochorion over gland mouths. These are not as highly specialized as in the pig (81).

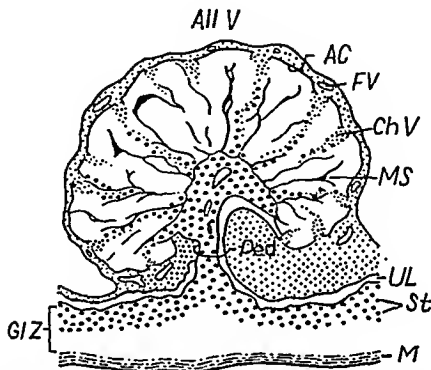


FIG. 6. Diagrammatic representation of a cow placentome. Note that the caruncle is convex, the fetal cotyledonary tissue surrounds, and its villi invade the caruncle. For key to lettering see legend to Fig. 1. By permission of H. W. Mossman (64) and the Carnegie Institution of Washington, Washington, D. C.

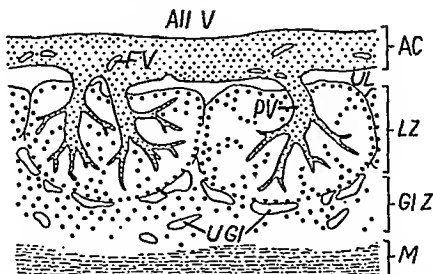


FIG. 7. Chorioallantois of the mare in the region of allantochorionic villi penetration ("cotyledon"). For key to lettering see legend to Fig. 1. By permission of H. W. Mossman (64) and the Carnegie Institution of Washington, Washington, D. C.

Wimsatt (81) divides the developmental periods of these areolae into three epochs. In the first (3rd to 7th week of gestation) areolae invaginate as thickened placodes of trophoblast to form shallow areolar crypts. In the second epoch (7th to 14th week) they are flattened by the stretching of the chorion and attain their definitive diameter. In the 3rd epoch (14th week to term), they become complexly folded.

B. *Perissodactyla*

Chorioamnionic folds are completed at about the 21st day of gestation in the mare. The allantois, like the amnion, is slow to develop as compared to the pig, cow, and sheep. It is only a small diverticulum at the 21st day (compare with sheep and pig), but by the 28th day it completely surrounds the amnion and nearly surrounds the yolk sac (31). By the 63rd day it has fused over the chorion and also fuses over the amnion to form a vascular allantoamnion (2).

In its early stages, the allantochorion is smooth and folded into sulci of uterine mucosa. About the 7th or 8th week, short, simple villi appear over the middle segment of the chorionic sac. At term these are present over the whole surface of the allantochorion, except at the opening of the Fallopian tubes opposite the os uteri internum, and over endometrial cups. Wislocki (84) believes these avillous areas are associated with relatively avascular zones of the chorion, while Mossman (64) argues that these are areas where stimulus of villi formation is lacking due to failure of the chorion to make contact with uterine mucosa. Bonnet (9) describes the arrangement of villi as cotyledons, since they form in groups and invade comparable areas of the uterine mucosa (Fig. 7). The villi are branched as in the sheep.

C. *Carnivora*

Development of the chorion, allantois and allantoeborion in the dog and cat is described in some detail by Bonnet (9), Bischoff (7), Duval (24, 25, 26), and in textbooks; however, no recent English monograph on either of these species is published. Wislocki and Dempsey (87) discuss in detail the histochemical reactions in the cat, but do not relate their tests to time of embryonic or membrane development.

Before the allantois fuses with the chorion (13½ days in the cat and 17 days in the bitch), the trophoblastic tissue of the vitellochorionic membrane establishes invasive villi (17, 38). Chorioamnionic folds separate the dorsal aspect of the embryo from the uterine lumen before the allantois fuses with the chorion and after the choriovitelline placenta is established. During its avascular period the chorion presumably acts as an absorptive membrane of histotrophe for fetal nutrition.

The allantois is large, as in the mare, sow, ewe, and cow, but encloses the amnion as in the mare. The yolk sac is pressed and held against the chorion by the allantois and does not separate until term (Fig. 2).

On the 18th day in the cat and 20th day in the dog, the allantois fuses with the chorion. Between the 13th and 18th day in the cat and 17th and 20th day in the dog, chorionic trophoblast is thickened but avillous. Allantochoionic villi form after fusion with the chorion and become increasingly complex as gestation proceeds.

Elongation of the allantochoionic sac takes place following formation of the definitive chorion. The extended portion of the chorion though lined by allantois remains avillous. Thus villi are restricted to a band of tissue about 45 to 50 mm. long girding the allantochoion in the region of the middle of the embryo. Villi of this region are complexly branched and invade deep into uterine mucosa (see Section VI).

VI. CHORIOALLANTOIC PLACENTA

Since the chorioallantoic placenta is the major structure through which the embryo and fetus derive nutrients from the maternal organism and through which the metabolites pass, its structure and function have been studied extensively. However, many problems concerning the physiology, morphology, and interrelationships between the maternal (uterine mucosa) and fetal (allantochoion) tissues during different stages of fetal development need yet to be resolved.

Chorioallantoic placentas have been classified in a number of ways. One classification is based on the distribution of allantochoionic villi, e.g., diffuse, zonary, cotyledonary, or discoidal. A second method is based on whether maternal tissue is lost, retained, or resorbed at parturition, such as deciduate, nondeciduate, and contradeiduate. The advantages and disadvantages of these schemes are discussed at length by Mossman (64) and Amoroso (2). Other methods have been derived; however, the most useful method of classification from the standpoint of the taxonomist and physiologist is that devised by Grosser (40, 41) and modified by Mossman (64). Grosser's scheme takes into account the degree of relationship between the fetal and maternal circulations. Embodied in this classification is an account of the number of tissue layers between the circulations and the extensiveness of invasion by allantochoionic villi into the uterine mucosa (Table I, Fig. 8).

Mossman (64) has used Grosser's classification effectively to show that deeper implantation, and invasiveness of allantochoion indicate greater specialization in a taxonomic sense. The physiological significance of this specialization has not been determined.

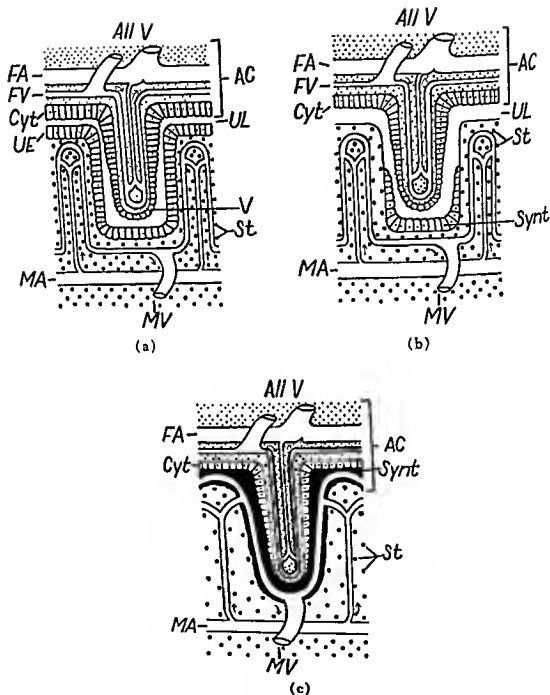


FIG. 8. Diagrammatic representations of tissue relationships between maternal and fetal tissues in the chorioallantoic placenta. (a) Epitheliochorial type. See also Table I. (b) Syndesmochorial type. (c) Endotheliochorial type. Syncytiotrophoblast (Synt) surrounds maternal capillaries. See Fig. 2. For key to lettering see legend to Fig. 1. By permission of H. W. Mossman (61) and the Carnegie Institution of Washington, Washington, D. C.

The sow and mare have an epitheliochorial type of placenta (the more primitive); the ewe, cow, and goat have a syndesmochorial type, while the carnivores (dog and cat) have an endotheliochorial type (Table I). The still more specialized hemochorial and hemoendothelial types, in which the maternal vascular endothelium is lost, are not discussed here since these types do not appear in domestic animals (Table I).

In our consideration of the placental types in domestic animals both the maternal portion and fetal contribution are discussed in this order.

A. Epitheliochorial Placenta

This placental type is present in the sow, mare, donkey, and camel of the domestic animals. The allantochorion of these animals remains external to the endometrium and becomes apposed to it in a simple way (Fig. 8). Thus, at least six layers of tissue, and potentially the uterine lumen and its secretions, separate maternal and fetal blood. The endometrial relationship is nondeciduate and the gross shape is diffuse.

The uterus of the sow, as in other mammals, is composed of three distinctly different tissue layers: the perimetrium (the outer connective tissue layer), myometrium (muscular layer), and endometrium (uterine mucosa) (Fig. 3). Although histological and morphological changes occur in the myometrium during gestation, changes in endometrium are more apparent and have been investigated more thoroughly.

Superficially, the mucosa of the sow uterus is transversely folded and the epithelium overlying this layer is composed of simple and non-ciliated columnar cells. Gland tubules, which extend through the mucosa to the myometrium, are lined with columnar ciliated epithelium. Although there are few gland tubules in the superficial layer, numerous fundic portions of the glands lie in the deeper layer and extend to the myometrium (16, 45).

Gland cells are characterized by basophilic cytoplasm and numerous mitochondria (88). Also, they are rich in phosphatase calcium, iron, and lipids; however, they contain little fat and no stainable glycogen (87).

A series of excellent photomicrographs by Amoroso (2) show that in early pregnancy the uterine endometrium is composed of shallow folds which become deeper and more complex in later gestation. Comparable allantochorionic ridges fit into the fossae of the uterine mucosa. Interlocking of allantochorionic and uterine folds together with tumescence of these tissues (through increased vascularity) is adequate to anchor the chorionic sac in the uterine lumen (45, 87).

The questions of how closely apposed allantochorionic trophoblast is to the endometrial epithelium has been a difficult one to resolve because fixation or mechanical disturbance may separate the tissues. Abramovich (1) describes an 80- μ thick layer of mucus between these tissues, but others do not support this finding (16, 45).

Epithelial cells lining the folds and ridges of endometrium are low cuboidal throughout gestation while trophoblast cells of the allanto-chorion change during pregnancy. Until the 3rd week these cells are cuboidal. They form a syncytium in the 4th week after which they become columnar. Later, the cells overlying allantochorionic ridges may become cuboidal, squamous, or syncytial, while cells in the fossae are columnar and have brush borders (for more detailed accounts of the histochemistry of the pig placenta, see Wislocki and Dempsey, 87). Goldstein (34) has shown that fetal capillaries extend into the trophoblast layer and pass between the epithelial cells of the allantochorion. Wislocki and Dempsey (87) suggest that even though fetal capillaries may come to the surface of the allantochorion, each is surrounded by a network of argyrophilic fibers (Table I); thus, the "epithelioendothelial" placental designation by Goldstein (34) in pigs is not valid. However, in early stages of gestation (3-7 mm. C-R) although not so evident in later stages, protoplasmic extensions from the cuboidal cells on the ridges of allantochorion extend between epithelial cells lining the fossae of the endometrium. These extrusions and the brush borders on columnar cells at the base of allantochorionic villi suggest a closer relationship between fetal and maternal tissue than is indicated by the epithelio-chorial classification (87).

The placenta of the mare like that of the sow is an epitheliochorial type, diffuse and nondeciduate. Its allantochorion is also in more intimate apposition to the uterine mucosa in some parts than others. This placenta shares its function with a choriovitelline placenta for a longer period of time, and attachment is completed relatively later in the mare than in the sow.

In early stages, the allantochorion follows the contours of the uterine mucosa which is transversely folded as in the sow. Villi develop in the middle part of the allantochorion during the 7th week of gestation and grow into fossae of the uterine mucosa. Villi of the mature placenta are long, with a highly vascular mesenchymal core, and are arranged in a circular pattern around gland mouths. The smooth circular area on the allantochorion at the base of the circularly arranged primary villi has been designated areolae. Groups of mature villi branch and fit into pockets (fossae) of mucosa which are shaped to fit these clumps. This

peculiar arrangement led Bonnet (9) to designate these as "cotyledons" (Fig 8)

Epithelium of the uterine mucosa underlying the allantochorionic villi is squamous to low cuboidal and beneath this crypt epithelium is a rich plexus of maternal capillaries (31) Elsewhere these cells are cuboidal or low columnar

The placenta of the mare can be divided morphologically into three layers in the region of the "cotyledons" the allantochorion with its mesenchyme and the base of the primary villi, a labyrinth, which includes the simple and interlocking branches of the allantochorionic villi lying in fossae and surrounded by ridges of uterine mucosa, and finally, the glandular layer of the uterine mucosa overlying a thin layer of dense connective tissue next to the muscularis (Fig 8)

According to Assheton (3) and others, the intervillous areas of the allantochorion function as an absorptive region for histiotrophic while the highly vascular villi are involved in absorption of hematotrophic

A peculiar specialization in the mucosa of the pregnant mare is the "endometrial cups" (15) Schauder (70) first described them as occurring between the 6th and 20th week of gestation and serving the function of supplying "uterine milk" Cole and Goss (15), Day and Rowlands (20a), and Clegg *et al* (13a), have shown that they contain a high titer of gonadotropin (Chapter 3)

The cups form in that part of the endometrium where the blood vessels from the umbilical cord branch and spread along the allantois. This constant relationship between the blood vessels of the allantois and the area of formation of the cups suggests that the developing placenta plays a role in the formation of the endometrial cups. They are formed in a semicircular area of the uterus and their size may range from several millimeters to 5 centimeters in diameter.

The endometrial cups form by modification of the endometrium in the gravid uterus (13a). In the early stages they are modified folds that are distinguished from other folds by their pale appearance and the increased size of the lumens of the necks of the uterine glands. Histologically, the cells lining the necks of the glands become polygonal and the cells of the stroma enlarge and become 'deciduallike'. As the cup develops the central portion is depressed, the edges are raised, and it is filled with a megakaryon containing degenerate epithelial cells, erythrocytes, polymorphonuclear leucocytes, and gonadotropin. In most cases secretion from the endometrial cup accumulates and causes a pouch to form in the allantochorion (Fig 9). The size and number of pouches

formed vary with individuals; in some cases the neck of the pouch may constrict and isolate the secretion from the uterine lumen.

Because of the relationship of the allantochorion to endometrial cups, some workers earlier suggested that the mare placenta might be an intermediate between the epitheliochorial and syndesmochorial type placentas. Until further work is done on other species, such as the donkey and zebra, the taxonomic position of these will remain unanswered.

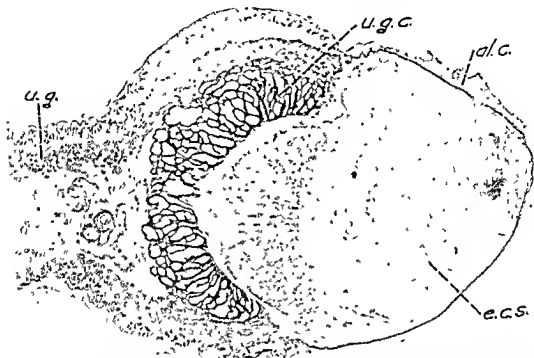


FIG. 9. Endometrial cup from mare (4) sacrificed on 105th day of pregnancy. Note the tremendous enlargement of the lumens of the uterine glands in the cup area. The cups are pendulous at this stage and represent a beginning stage in the formation of an allantochorionic pouch. al.c., allantochorion; e.c.s., endometrial cup secretion; u.g.c., uterine glands in cup area, u.g., uterine glands outside of cup area. Magnification: $\times 6$.

B. Syndesmochorial Placenta

This type of placenta is found in ruminants and involves the disappearance of uterine epithelium over restricted or, in some cases, large areas, thus allowing allantochorionic villi to come into contact with uterine stroma. Five tissue layers in this placental type lie between fetal and maternal circulation (Fig. 8). The gross form of this type may be cotyledonary or multiplex, with deciduate and nondeciduate areas. In sheep the placenta has contradeciduate areas.

Any discussion of this type of placenta must center around the cotyledon, its structure, and formation, as well as the ultimate relationship between maternal and fetal blood (Figs. 5, 6, and 8).

Specialized circular areas of uterine mucosa in the uterine horns of the ewe, cow, and goat project into the uterine lumen. These projections are properly designated caruncles or cotyledonary burrs (maternal cotyledons). If allantochorionic trophoblast becomes specialized over these areas and forms villi which invade the caruncle, the resulting structure is designated a placentome (22) (Figs. 5 and 6). Invading groups of allantochorionic villi are the cotyledon (fetal cotyledon). In recent literature it is a common practice to refer to the placentome as the cotyledon. This writer would prefer to retain the classic nomenclature so that the reader will have clearly in mind the structure being discussed.

The number of caruncles in different ruminants varies. The goat has 160-180 (polycotyledonary), roe deer 4 or 6 (oligocotyledonary), while the sheep with 88-96 and the cow with 70-120 are intermediate (2, 14, 67). These are arranged in four irregular rows in the gravid and non-gravid horn of sheep (14) and, in the cow, as a series of four rows in the body, three in the middle of the uterine horns, and two in its extremities (42).

Since fetal cotyledons necessarily form over the caruncles, they, too, will tend to be in an alignment; however, not all caruncles are invaded by chorionic villi. Thus the placentome number is usually less than the number of caruncles.

Between the specialized areas (caruncles) of the uterine mucosa are the intercaruncular spaces. The caruncles increase in size during pregnancy and are largest in a region dorsal to the embryo. Shapes of the caruncles differ in the various ruminants. They are nearly closed concave in sheep (Fig. 5), open concave in the goat, and convex in the cow (Fig. 6). The caruncles are nonglandular and lined with tall columnar cells with basal nuclei. Epithelial cells of the intercaruncular area are somewhat lower than over the caruncles. According to Ellenberger and Baum (29), these cells are ciliated in the young animals but this characteristic is not present in the mature animal. Glands opening into the uterine cavity are long, coiled, tubular, and branched at their base. They decrease in number toward the cervix (2).

Pigments are often found in the uterine mucosa and oviducts of ewes and may even be present in fetal lambs. This pigmentation, according to Grant (35), is due to the presence of "true" melanoblasts. No functional significance has been attributed to them.

The core of caruncles is composed of stroma in which numerous maternal capillaries enter at right angles to the endometrium.

Thickening of allantochorionic trophoblast overlying the caruncles is the first indication of cotyledon formation in sheep (17th day). This is followed by degeneration of uterine epithelium which, according to Assheton (3), does not regenerate but is replaced eventually by binucleate cells of fetal origin. Up to this time and until the 31st day the allantochorion is held in position against uterine mucosa by pressure of fetal fluids. Fetal villi begin to penetrate the caruncles at the 31st day and by the 44th day convex cotyledons fit into the concavities of the caruncle to form the initial placentome. The definitive placentome is completed by the 78th day, at which time the peripheral borders of the caruncle curl inward, thus binding tightly the enclosed cotyledon (Fig. 5).

In the cow, according to Hammond (42), invasion of villi from the allantochorion begins at about 30 days postcoitus and is completed between the 3rd and 4th months. Before the end of the 2nd month uterine epithelium of the entire caruncle is gone and chorionic villi begin to branch and penetrate into fossae and surround ridges of maternal tissue of the caruncle. By 90 days the cotyledon is fully mature; attempts to separate the cotyledon and caruncle result in tearing of fetal and maternal tissue.

The method by which uterine tissue is destroyed during invasion of allantochorionic villi is unknown. In the ewe and cow, binucleate cells of chorionic origin migrate into uterine stroma and form a layer under the uterine epithelium. These cells reportedly engulf maternal epithelium (64).

Along with degeneration and subsequent loss of uterine epithelium in the caruncular region, a similar process occurs in the intercaruncular epithelium, except around gland mouths. Gland secretion may prevent close adherence of trophoblast in these regions and thus protect gland epithelium from destruction by binucleate cells of fetal origin (2, 44).

Regeneration of uterine epithelium may occur in both the cow and ewe over most of the intercaruncular area. This phenomenon raises the unanswered question as to the origin of these cells. If, as Hammond (42) states, they are not true epithelial cells "but consist of connective tissue, plasma, or lamella cells . . . which tend to congregate on the abraded surface" then they are of maternal origin and thus would constitute an epitheliochorial placental type. Regardless of origin of these cells, there would be at the tips of the villi six layers of tissue between

maternal and fetal circulation rather than five (Table I). This whole problem needs further investigation (64).

C. *Endotheliochorial Placenta*

In this placental type, allantochorionic epithelium is in apposition to endothelium of maternal vessels. It is the characteristic placenta of Carnivora but is also present in species of other orders (Insectivora, some chiropterans, and sloths of Edentata).

Prior to implantation, the mucosa of the dog and cat becomes extensively folded and uterine gland lumina dilate. Associated with these changes is a thinning of uterine epithelium and increased secretory activity by the glandular epithelium. As a result of glandular growth the uterine mucosa can be divided into two zones: an outer compact and an inner spongy zone (Fig. 2). The compact zone is composed of many glands and tubules which are crowded together thus constricting stroma while the spongy zone results from widening of the lumina of the more sparse fundic portions of deeper glands. These glands extend to the muscularis (44). The glandular secretions are less fluid than in ungulates and degenerate glandular epithelium is more concentrated in the histotrophe of the dog and cat (55).

Invasion of the mucosa by allantochorionic villi is restricted to a band in the middle of the implantation chamber. In this region uterine epithelium is destroyed, and solid villi penetrate into the exposed stroma at about the 13th or 14th day in the cat (39) and 16th to 17th day in the greyhound bitch (2). Villi also penetrate into gland lumina and crypts as well as between epithelial cells that remain (24, 44, 75).

Uterine epithelial cells during implantation lose their outlines and form a symplasma consisting of a homogenous mass of protoplasm in which lie fragmented nuclei (8). When broadly interpreted, the symplasma consists of glandular epithelium, connective tissue cells, as well as extravasated blood. Bonnet (8) believes that syncytium should remain as a designation of an active protoplasmic condition; thus, in Carnivora, uterine epithelium during implantation is symplastic rather than syncytial.

Enlargement and proliferation of mucosal cells (decidual reaction) and enlargement of maternal blood and lymph vessels are associated with penetration of villi.

After fusion of allantois to the chorion (18th day in the cat and 20th day in the dog) primary villi of the chorion begin rapid growth soon surpassing choriovitelline growth (Section II) and penetrate deeply into uterine mucosa. The primary villi give off secondary and tertiary

branches when allantoic vessels grow into the core of the villi. In its path of penetration the uterine symplasma is absorbed presumably by the trophoblastic cells. The villus epithelium becomes syncytial (syncytiotrophoblast) except at the tips where it remains cellular. These villi penetrate everywhere into the mucosa, in which all but maternal capillary endothelium disappears (24, 44).

By the end of the first month, the placenta is mature and from this date changes are merely elaborations of the already definitive placenta. In its maturation, villi penetrate through the more superficial compact zone and thence into the spongy zone of the uterine mucosa (Fig. 2). Symplasma forms ahead of the villous tips and is subsequently absorbed. Villi finally come to lie in the interglandular septa of the spongy zone. The more superficial and complex portion of the labyrinthine zone of the placenta forms as a result of villi with their mesenchyme developing as a network of fetal tissue around the maternal vessels (44).

Near term, the placental labyrinth in cats extends to the deep glandular or spongy zone of the uterine mucosa (see Fig. 15.32 Amoroso, 2); thus the labyrinth comprises most of the placental thickness. In dogs, the spongy layer is about one-third the height of the placental labyrinth near term.

The labyrinth of the dog and cat differ further in that the labyrinth in the dog is lobular and its lamellae are elaborately branched but are disposed more or less vertically; in the cat, each lobule is formed from the adjacent trophoblastic layers of two primary villi enclosing a maternal matrix in which are situated the relatively thin-walled maternal capillaries.

A characteristic feature of the placenta in Carnivora is the presence of a hematoma formed from extravasation of maternal blood either at the border or central portion of the placenta (Fig. 2). In dogs, a green pigment "uteroverdin" appears in the marginal hematoma. This pigment, the result of hemoglobin breakdown, has resulted in the use of the term "bordures vertes" (green border) in naming the hematoma of dogs (24-26). In the cat, the green pigment appears early but in later stages of gestation the hematoma takes on a brown color—thus the brown border. Hemorrhages occur more irregularly in the cat than in the dog (44).

Depressions in the allanto chorion (pockets) overlie extravasated maternal blood. Some workers believe these pockets result from the pressure of the extravasated maternal blood pushing the allanto chorion away from the mucosa. Villi from the allanto chorion dip into these pockets and absorb its fluid constituents and engulf, by phagocytosis,

the larger particles such as erythrocytes (44). Cells lining these villi are columnar.

Vesicles in the allantochorion lined by trophoblast are found in the cat. As they contain a brown coagulum, these vesicles have been termed supernumerary "pockets." Because they are vascular and have vacuolated epithelium similar to the villi of the brown border (65), they constitute an apparatus obviously similar to the marginal hematoma.

Structures in the paraplacental region of the chorion, called "rosettes," are light-staining, raised areas related to the mouths of uterine glands. These areas may vary from single to many swollen, columnar trophoblast cells. They are present in the dog's chorion until term, and doubtless correspond to irregular areolae of the pig and other ungulates (2). A detailed discussion of the histochemistry of the placenta of the cat and dog are found in papers by Kolster (55), Wislocki and Key (88), and Wislocki and Dempsey (87).

VII. AGING AND FETAL DEVELOPMENT

With the exception of the pig, studies on prenatal development of farm animals have received very little attention. The need for such studies becomes apparent when one wishes to evaluate normal variations, to make comparisons between species, and to understand better the specific effects of environmental factors in the development of embryos or fetuses *in utero*. In these studies, it is often necessary to estimate accurately the ages of embryos or fetuses, even though fertilization time may not be known.

Following fertilization of an ovum, a series of predictable events follow in an orderly sequence which culminates in a fully formed organism characteristic of its parental species. As the embryo develops from the one-celled stage to its complex fetal form, the number of structural arrangements which could be used to characterize each period of development increases progressively. If one were to classify each stage of development according to each morphological change, the number of stages would become nearly infinite and thus of little value to the worker. A satisfactory method for classifying embryos or fetuses of domestic animals as to age or stage of development has not yet been derived. To be of maximum value, descriptions and measurements must be made according to standard methods; parameters set for each stage should be useful to the comparative and experimental embryologist as well as the morphologist.

Mall (60) realized the disadvantages of aging and staging embryos on the basis of length, as indicated by his statement, "In the course

of time it will be possible to arrange human embryos in stages, thus permitting a comparison of the structures of embryos of the same stage." He was at that time of the opinion that embryos could be arranged in stages based on their external form. Streeter (76), with new data and with a more complete series of human embryos, attempted to bring Mall's work to conclusion. At this time Streeter introduced the term "horizon" to emphasize the importance of thinking of the embryo as a living organism which progresses from the smaller and simpler to the larger and more complex. He divides prenatal development into two periods, embryonic and fetal. The embryonic period, extending from the single-celled egg to the time of eyelid closure, is divided into 23 stages. Stages I-VII describe the ovum, cleavage, blastocyst, implantation, and nature of the yolk sac and chorion; stages VIII-XIII describe the early differentiation of the germ disk, somite number, differentiation of external structures (ectodermal derivatives), as well as differentiation of tissues and organs; finally, stages XIII-XXIII describe the completion of embryonic development. A detailed exposition of the individual stages is not possible in the space allotted for this chapter.

Following eyelid closure, the embryo, for convenience, may be considered a fetus until parturition. According to Streeter (76), "Weekly increment in growth and weight is large enough to provide an adequate index of relative development," in humans. The validity of this view, and whether other criteria, such as hair follicle development, fusion, and ossification rate of bones, might be used more effectively in aging fetuses to term must await further investigations.

Winters *et al.* (82, 83) and Green and Winters (37) divide the prenatal period of the ewe and cow, respectively, into the ovum, embryonic, and fetal periods. The ovum period extends from fertilization to attachment; the embryonic period comprises the interval during which the major tissues, organs, and the systems are formed; and the fetal period includes the intrauterine time after the organs are well formed. This general classification, used in domestic animal embryology, reflects a lack of exhaustive studies in this field. In other words, the necessary criteria are lacking to define precise developmental periods in domestic animals to arrange embryos or fetuses according to age.

Before beginning a study on the growth or aging of embryos or fetuses, reference should be made to the work on humans (48, 72, 76-78), on frngs (73), on the chick (50), on sheep (5, 14), on the cow (42), and on the dog and cat (24-26).

Since development of the ovum, embryo, and fetus are similar in all eutherian mammals, one can follow the excellent work on pig embryol-

ogy by Patten (66a) for an approach to the various "horizons" in embryonic and fetal development which may be useful in staging and aging studies of domestic animals

Because of their convenience, linear and circumferential measurements used extensively in postnatal growth studies have likewise been applied to investigations on prenatal growth, they have also been used as a criterion of embryonic or fetal age. Unfortunately, measurements taken by different workers and in different laboratories have been too variable to be of value elsewhere. Embryos of comparable size and the same age may also be in different developmental stages (see Section V, A). In studies necessitating the determination of the age at fetal death *in utero*, the embryo or fetus undergoes partial resorption and degeneration. Thus, the length, weight, and volumetric measurements are unacceptable as criteria of age. Consequently, it becomes necessary to use structures that are resorbed at a slow rate. In this writer's experience, some of these would be cartilage and ossification centers (5), pinna of the ear, stage of appendage differentiation (such as tail and limb buds). In the early embryo, gill slits and the state of eye and ear differentiation are recognizable in early stages of resorption. We do not know to what extent external and internal structures are retained, or to what extent fetal membranes degenerate or develop following fetal death. This field remains to be explored.

In some experiments on pregnant animals, fetal size and birth weights have been used as criteria of the experimental effects on the embryo and fetus. However, MacDowell *et al* (58), using pure strains and accurately determined gestation ages in rats, found a considerable variation in weights and sizes of fetuses. Earlier, Fitch *et al* (32) found a significant difference in birth weight, size, and ratio of calf to maternal weight in four different milk breeds of cattle. Embryo and fetal growth within the usual experimental conditions are independent of the extra-uterine environment, however, maternal maturity (in age) is related to an increase in fetal and parturient weight and size in cows (27, 28, 32), in rats (22, 53), in pigs (57, 71), and in sheep (21). A number of investigations have shown that as litter size increases there is a decrease in fetal and parturient weight and length in sheep (66) and in pigs (71). Further, it has been shown that the birth weight of the male exceeds that of the female in humans (36), in sheep (21), in cows (32), and in pigs (71). This sex difference has been shown to express itself as early as the 2nd month in cattle (4, 52). It becomes apparent that use of weight or size as an index of fetal age must take into account a number of factors: breed and strain, maternal age, and litter size, as

well as the genetic effects of the sire. Evidence is accumulating which shows that season of breeding may be correlated with fetal weight (71).

The existence of adequate aging standards for embryos or fetuses of the various domestic animals would make available for studies of prenatal growth the abundance of material from the various slaughterhouses. Our present knowledge of prenatal growth in mammals has been gained by studies on small laboratory animals. This work confirms the generally held view that fetal growth does not differ qualitatively from postnatal growth (23, 58, 74).

Prenatal growth studies in domestic animals are scattered in the literature and are based on inadequate material. Most workers have not defined carefully their techniques of measurements and have used weight and length of the embryo or fetus as criteria of age. Thus, a comprehensive table on prenatal growth in domestic animals cannot be compiled at this time.

Enough work has been done, however, to indicate trends of growth in weight, length, and some other dimensions.

As the fetus ages (time) there is an orderly addition of new cells and tissues, and as a result of the growth of its parts the fetus changes in its dimensions. As the dimensions increase in size, the weight must necessarily increase. Consequently, one is not surprised to find a relationship between age, weight, and changes in length of the various dimensions (Fig. 10).

Curson and Malan (18), Winters and Fueffel (83), and Cloette (14) show that when weight is plotted against age in sheep fetuses the result is a smooth logarithmic curve. This has been shown to be true in other species as well: human (48), guinea pig (23), and mouse (58).

Brody (11) (on rats, guinea pig, chick, and man) and Kislovsky and Larchin (54) (on cattle) have indicated that there are "breaks" in fetal growth rates. However, these workers have been criticized for their view because of insufficient quantitative data (14, 83).

Cloette (14) states, "It is possible to express all fetal growth-rates by the same general formula, the constants of which have only to be adjusted for each species." He used the following formula on fetuses of Merino sheep to designate the instantaneous relative growth rate (K) in weight:

$$K = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

where W_2 and W_1 are the fetal weights at the ages T_2 and T_1 days, respectively. Application of this formula to data collected on cow fetuses

by Rorik (67), Bergmann (6), Hammond (42), and Winters *et al.* (82) results in an age-weight curve similar to that of sheep (14).

A number of linear and circumferential measurements on fetuses have been made with varying success in an attempt to describe prenatal growth and to relate these to age. Some of these are illustrated in Fig. 9. Most of these are modified from the preferred measurements used on human fetuses as first described by Mall (60) and later elaborated on by Schultze (72).

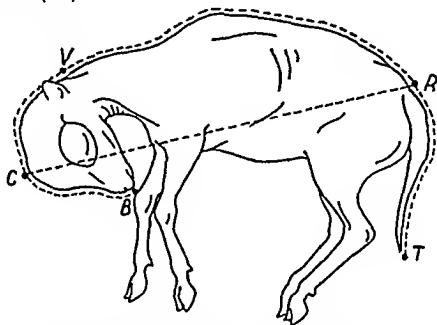


FIG. 10. Sheep embryo illustrating some dimensions used for aging and growth studies. CR (crown-rump); CVR (curved crown-rump), VR (vertebral column length), VRT (vertebral column length, including the tail), BCVRT (total length).

In primates the crown-rump (C-R) or sitting height is the basic linear measurement. Because of the morphological difference between primates and other mammals (such as the elongated neck and tail) various modifications of this measurement have been devised. Some of the more common are poll to base of tail, forehead to base of tail, nose-anus length (18, 43). Use of these measurements have been criticized, since neck length may be extremely variable and easily changed by manipulation of the fetus (14, 22). Cloette (14), through use of careful and standard techniques applied to Merino sheep fetuses, showed that the curve of an instantaneous growth rate (crown-rump) is smooth and without "breaks" and similar to the weight curve. He further found the coefficient of variation to be 7.51% for this measurement when compared to age. Winters *et al.* (82) found a similar curve for bovine fetuses.

Curson and Malan (19) introduced a curved crown-rump dimensional measurement which followed the dorsal contours of the body. These workers were aware that this measurement gave a less satisfactory "fit" than did the straight crown-rump measure. Cloette (14) also found

this to be true in his work on Merino sheep (coefficient of variation, 10.34).

A modification of Mall's (60) vertebral column (V.C.) length (length of the entire vertebral column, including the tail) proved to be the most satisfactory measurement in Cloette's work. He used the formula:

$$\log_e \text{V.C.} = 4.3948 \log_e \text{age} - 0.2949 (\log_e \text{age})^2 - 10.4883$$

When tested for significance, this gave a positive result at the level of $P = 0.01$ with a coefficient of variation at 3.8%. When sufficient data are plotted and represented by curves of the second degree, there are no breaks. Winters and Fueffel (83) and Winters *et al.* (82) found that the contour length (a measure from the tip of the nose to the end of the tail following the body contour) was the most satisfactory measure of growth from the somite through fetal stages. These workers did not apply statistical tests to their measurements, presumably because they lacked adequate numbers. Both groups conclude that embryos with similar measurements may be of a different age and that weight is not necessarily indicative of different degrees of differentiation. Winters *et al.* (82) further conclude "Because of the inadequacies of measures such as weight, length, volume . . . , appearance is the most valuable single measure for comparison of prenatal specimens." Cloette (14), following Scammon (69), constructed a normograph for Merino sheep from which age can be determined with reasonable accuracy by use of a number of dimensional measurements.

In our review of the literature, it became more and more apparent that, to date, we lack adequate quantitative data for a detailed account of the prenatal existence of domestic animals. However, papers cited above may act as guides to future investigations.

REFERENCES

1. Abramovich, G. E., *Anat. Record* **33**, 69 (1926).
2. Amoroso, E. G., in "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), p. 127. Longmans, Green, New York, 1952.
3. Assheton, R., *Phil. Trans. Roy. Soc. London Ser. B* **193**, 143 (1906).
4. Beer, G., *Jahresber. Vet. Med. (abstr.)* **45**, 237 (1925).
5. Benzie, D., *Brit. Vet. J.* **107**, 3 (1951).
6. Bergmann, R., *Arch. wiss. u. prakt. Tierheilk.* **47**, 292 (1922).
7. Bischoff, T. L. W., "Entwicklungsgeschichte des Hundeeies." F. Vieweg H. Sohn, Braunschweig, Germany, 1845.
8. Bonnet, R., *Anat. Hefte* **20**, 323 (1903).
9. Bonnet, R., "Lehrbuch der Entwicklungsgeschichte." P. Parey, Berlin, 1907.
10. Bremer, J. L., *Am. J. Anat.* **19**, 179 (1916).
11. Brody, S., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 97*, 1 (1927).
12. Brody, S., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 98*, 1 (1927).
13. Chang, M. G., *Anat. Record* **113**, 143 (1952).

- 13a Clegg M T, Boda, J M, and Cole, H H, *Endocrinology* 54, 448 (1954)
- 14 Cloette, J H L, *Onderstepoort J Vet Sci Animal Ind* 13, 417 (1939)
- 15 Cole, H H, and Coss, H, "Essays in Biology," No 107 Univ of Calif Press, Berkeley and Los Angeles (1943)
- 16 Corner, G W, *Contribs Embryol Carnegie Inst* 13, 117 (1921)
- 17 Courier, R, and Cros, G, *Compt rend soc biol* 111, 787 (1932)
- 18 Curson, H H, and Malan A P, *Onderstepoort J Vet Sci Animal Ind* 4, 481 (1935)
- 19 Curson, H H, and Malan, A P, *Onderstepoort J Vet Sci Animal Ind* 7, 261 (1936)
- 20 Davies, J, *Am J Anat* 91, 263 (1952)
- 20a Day, F T, and Rowlands, I W, *J Endocrinol* 5, 1 (1947)
- 21 Donald, H P, and McClean, J W, *New Zealand J Sci Technol* 17, 497 (1935)
- 22 Donaldson, H H, "Memoirs of the Wistar Institute of Anatomy and Biology" Philadelphia, Pennsylvania 1924
- 23 Draper, R L, *Anot Record* 18, 369 (1920)
- 24 Duval, M, *J Anat Paris* 29, 249, 426, 663 (1893)
- 25 Duval, M, *J Anat Paris* 30, 189, 649 (1894)
- 26 Duval, M, *J Anat Paris* 31, 38 (1895)
- 27 Eckles, C H, *Missouri Univ Agr Expt Sto Research Bull No* 35 (1919)
- 28 Eckles, C H, *Missouri Univ Agr Expt Sto Research Bull No* 36 (1920)
- 29 Ellenberger, W, and Baum, H, 'Handbuch der Vergleichenden Anatomie der Haustiere" Hirschwald, Berlin, 1921
- 30 Eschricht, D F, cited in Heuser (45)
- 31 Ewart, J C, *Trans Roy Soc Edinburgh* 51, 287 (1915)
- 32 Fitch, J B, McGilliard, P C, and Drum, G M, *J Dairy Sci* 7, 222 (1924)
- 33 Gersh, I, *Contribs Embryol Carnegie Inst* 26, 33 (1937)
- 34 Goldstein, S R, *Anot Record* 34, 25 (1926)
- 35 Grant R, *Vet J* 89, 271 (1933)
- 36 Green, W W, *Am J Vet Research* 7, 395 (1946)
- 37 Green, W W, and Winters, L M, *Minn Univ Agr Expt Sto Tech Bull No* 169, 1 (1945)
- 38 Griffiths W F B, and Amoroso, E C, *Vet Record* 51, 1279 (1930)
- 39 Cros, C, Thèse Médecine, Alger, Algiers, 1936
- 40 Crosser, O, Frühentwicklung Eihautbildung und Placentation des Menschen und der Säugetiere" Bergmann, München, 1927
- 41 Crosser, O, *Verhandl Anat Ges, Jena* 81, 15 (1930)
- 42 Hammond, J, 'Reproduction in the Cow" Cambridge Univ Press, London, 1927
- 43 Hammond, J, ed, Progress in the Physiology of Farm Animals," p 793 Butterworths, London, 1957
- 44 Heinrichs, C, *Anat Hefte* 50, 115 (1914)
- 45 Heuser, C H, *Contribs Embryol Carnegie Inst* 19, 229 (1927)
- 40 Heuser, C H, and Streeter, C L, *Contribs Embryol Carnegie Inst* 20, 1 (1929)
- 47 Huggett A St C and Hammond, J, in 'Marshall's Physiology of Reproduction" (A S Parke, ed), p 312 Longmans, Green London 1952
- 48 Jackson, C M, *Am J Anat* 9, 119 (1909)
- 49 Janssen A, *Med Vet Diss Hannover, Germany*, 1933

50. Jenkinson, J. W., "Vertebrate Embryology." Oxford Univ. Press, London, 1913.
51. Jordan, H. E., *Am. J. Anat.* 19, 227 (1910).
52. Keller, K., *Wein. tierärztl. Monatsschr.* 7, 137 (1920).
53. King, H. D., *Anat. Record* 9, 213 (1915).
54. Kislowsky, D. A., and Larchin, B. A., *J. Agr. Sci.* 21, 659 (1931).
55. Kolster, R., *Anat. Hefte* 16, 794 (1908).
58. Lillie, F. R., "Development of the Chick" (Rev. by H. L. Hamilton). Holt, New York, 1952.
57. Lowrey, L. G., *Am. J. Anat.* 12, 107 (1911).
58. MacDowell, E. C., Allen, E., and MacDowell, C. G., *J. Gen. Physiol.* 11, 57 (1927).
59. Malan, A. P., and Curson, A. P., *Onderstepoort J. Vet. Sci. Animal Ind.* 7, 239 (1936).
60. Mall, F. P., in "Manual of Human Embryology" (F. Keibel and F. P. Mall, eds.), p. 180. Lippincott, Philadelphia and London, 1910.
61. Markee, J. E., *Anat. Record* 88, 329 (1944).
82. Markee, J. E., and Hinsey, J. C., *Proc. Soc. Exptl. Biol. Med.* 31, 267 (1933).
83. Melton, A. A., Berry, R. O., and Butler, O. D., *J. Animal Sci.* 10, 933 (1951).
64. Mossman, H. W., *Contribs. Embryol. Carnegie Inst.* 26, 129 (1937).
85. Mossman, H. W., *Proc. Zool. Soc. London Ser. B109*, 373 (1939).
88. Murrar, J. A., *J. Agr. Sci.* 11, 258 (1921).
- 86a. Patten, B. M., "Embryology of the Pig." Blakiston Div., McGraw-Hill, New York, 1948.
67. Rorik, H. H., *Arch. wiss. u. prakt. Tierheilk.* 33, 421 (1907).
88. Rowlands, I. W., and McPhail, M. K., *Quart. J. Exptl. Physiol.* 26, 109 (1936).
69. Scammon, R. E., *Anat. Record* 68, 221 (1937).
70. Schauder, W., *Arch. Anat. u. Physiol. Leipzig* 192, 259 (1912).
71. Schneider, H., Inaugural Dissertation, Leipzig, 1938.
72. Schultze, O., *Contribs. Embryol. Carnegie Inst.* 20, 213 (1929).
73. Shumway, W., *Anat. Record* 76, 139 (1940).
74. Stotsenberg, J. M., *Anat. Record* 6(1), 667 (1915).
75. Strahl, H., *Anat. Hefte* 31, 199 (1906).
76. Streeter, C. L., *Contribs. Embryol. Carnegie Inst.* 30, 211 (1942).
77. Streeter, G. L., *Contribs. Embryol. Carnegie Inst.* 32, 133 (1948).
78. Streeter, G. L., *Contribs. Embryol. Carnegie Inst.* 33, 149 (1949).
79. Streeter, G. L., *Contribs. Embryol. Carnegie Inst.* 34, 165 (1951).
80. Turner, W., *J. Anat.* 10, 127 (1875).
61. Wimsatt, W. A., *Am. J. Anat.* 87, 391 (1950).
62. Winters, L. M., Green, W. W., and Cornstock, R. E., *Minn. Univ. Agr. Exptl. Sta. Tech. Bull. No. 336* (1942).
83. Winters, L. M., and Fueffel, G., *Minn. Univ. Agr. Exptl. Sta. Tech. Bull. No. 118* (1936).
84. Wislocki, G. B., *Biol. Bull.* 65, 80 (1933).
85. Wislocki, G. B., *Anat. Record* 63, 183 (1935).
86. Wislocki, G. B., and Bennet, H. S., *Am. J. Anat.* 73, 335 (1943).
67. Wislocki, G. B., and Dempsey, E. W., *Am. J. Anat.* 78, 1, 161 (1946).
88. Wislocki, G. B., and Key, J. A., *Contribs. Embryol. Carnegie Inst.* 13, 103 (1921).
69. Wright, P. L., *Anat. Record* 83, 34 (1942).
90. Zietzschmann, O., "Lehrbuch der Entwicklungsgeschichte der Haustiere." Schoetz, Berlin, 1923.

CHAPTER 14

Endocrine Mechanisms During Pregnancy

HUBERT R. CATCHPOLE

	<i>Page</i>
I. Introduction	470
II. General Endocrine Mechanisms in Pregnancy	471
A. Maternal Endocrine Functions	471
1. The Ovaries of Pregnancy	471
a. Endocrine Role of Estrogen	472
b. Endocrine Role of Progesterone	473
c. Nidation, Pseudopregnancy	473
d. Endocrine Role of Relaxin	474
e. Maintenance of Pregnancy	474
2. The Pituitary Gland of Pregnancy	475
a. Biological Actions	475
b. Role in Maintenance of Ovary and of Pregnancy	477
c. Relation to Other Endocrine Glands of Mother	477
3. Other Endocrine Glands in Pregnancy	478
a. Thyroid	478
b. Parathyroids	478
c. Adrenal Glands	478
d. Posterior Pituitary Gland	479
4. Hormone Levels in Pregnancy	479
a. Steroid Hormones	480
(i) Estrogens	480
(ii) Progesterone	483
(iii) Adrenal Cortical Steroids	486
b. Gonadotropic Hormones	487
(i) Chorionic Gonadotropin; Equine Gonadotropin	487
(ii) Gonadotropins in Other Animals	489
(iii) Prolactin (Luteotropin)	489
c. Relaxin	489
d. Oxytocin	489
5. Growth and Involution of the Uterus	489
6. The Mammary Glands in Pregnancy	490
B. Placental Endocrine Functions	491
1. Estrogens	492
2. Progesterone	492
3. Adrenal Cortical Steroids	492
4. ACTH, Thyrotropin	493
5. Relaxin	493
6. Gonadotropins	493
a. Chemistry and Biology of the Gonadotropins of Pregnancy	493
G. Endocrine Functions of the Fetus	494
1. Fetal Gonads	495
2. The Fetal Thyroid	496
3. The Fetal Adrenal	496

	<i>Page</i>
III. Special Aspects of Pregnancy in Domestic Animals	497
A. Mare	497
1. Equine Gonadotropin and the Ovaries	497
2. The Equine Placenta	498
3. The Fetal Gonads	499
B. Cow	499
1. Cervical Mucus	499
2. Nidation	499
3. The Corpora Lutea and Maintenance of Pregnancy	500
4. Progesterone and Ovulation	500
5. Relaxin and the Cervix Uteri	500
C. Goat	500
1. Maintenance of Pregnancy	500
D. Sow	500
1. Relaxin in the Ovary	501
E. Ewe	501
1. Estrus in Pregnancy	501
2. Maintenance of Pregnancy	501
3. Steroid Excretion	501
4. Milk Ejection	501
5. Blood Progesterone ..	501
References	501

I. INTRODUCTION

Pregnancy implies the complex of events that supervene from the time an ovum is fertilized to the expulsion of the fetus and its membranes. It usually comprises a lengthy time span in the economy of the animal. In some aspects it engenders mutually antagonistic reactions between mother and fetus, but in most others it appears as a process in the highest degree adaptive in kind, enforcing on the joint organism its own states of equilibrium or homeostasis. These adaptations tend to be mediated by endocrine secretions, even where critical stages involve triggering by the nervous system.

Pregnancy presupposes fertility: the production of ova; the ingress of competent sperm with successful fertilization, and an anatomically normal genital tract. The number of ova shed is largely characteristic of a species, though it may be modified by treatment with gonadotropic hormones (172). In the rat (35) and bovine (164, 185, 196), superovulation is readily induced. When numerous ova are received into the uterus, they are at first randomly scattered by uterine agitation; then the developing blastocysts are spaced out evenly by a mechanism related to local distention in a uterus under the influence of progesterone (21).

Not infrequently, the number of fetuses that develop to term is less than the number of ova shed. Fetal wastage was studied in the wild

rabbit (23), where some 10% of ova are lost before implantation and not less than 35% of the litters that survive implantation are lost *in toto*. Embryos are also lost in litters that survive. There is comparable pre-natal mortality in the large litters of the pig (113, 156) and in the sheep (59), and certainly frequent outright loss where one fetus is normally produced. Complex genetic, nutritional, and hormonal factors are probably involved.

During pregnancy, the uterus progressively adapts to the growing fetuses with their membranes and fluid contents. The basis of this adjustment is obscure, although the thesis that stretching by the conceptus is the stimulus for growth has been proposed (162). That this process is at least conditioned by hormones is shown by the growth undergone by a sterile uterine horn in the environment of a fertile one.

At a predetermined time in development, fetus and placenta are born, and the uterus thereupon involutes (Section II.A.5.). The immediately succeeding period of lactation, with suckling or milking, is one of lowered fertility, with irregularity of estrus and frequent failure of ovulation, which have been well observed in domestic forms (123, 186).

The material and energetic changes that occur between mother and fetus were the subject of Newton's classic account (143). The objective of the present chapter is to examine the endocrine correlates of pregnancy. Section II deals with mechanisms which many animals appear to have in common, projected where necessary from common laboratory forms to domestic animals. Section III takes up some aspects in which the domestic forms have been the main focus of interest. While no absence of generality is necessarily implied by this division, it has become apparent that the same ends may be served by disparate endocrinological means.

A largely descriptive account of hormonal relationships should be tempered to the goal of defining hormonal "action" (114); this is attempted in a modest, if personal, way. The article of Hechter (85) provides a further point of view and an excellent critique of current hypotheses.

II. GENERAL ENDOCRINE MECHANISMS IN PREGNANCY

A. Maternal Endocrine Functions

1. The Ovaries of Pregnancy

In the presence of fertilized and implanting ova, changes occur in the ovary which in general define its character for the remainder of the gestation period. Corpora lutea, which normally would regress, are stabilized as corpora lutea of pregnancy; these may be considerably

larger than the corpora of the estrous cycle, although the individual cells are not greatly altered (136). Follicles which would have been expected to enlarge with the onset of another cycle are inhibited, and with them, estrus itself. These statements are subject to quantitative modification. The life span of the corpus luteum of pregnancy may cover the entire gestation period or only a part of it (see reference 177, Table 103). Suppression of the estrous cycle may be complete, or one or more periods of heat may recur. In the mare, the initial, generally single, corpus luteum is short-lived but a multiple second crop appears later.

The precise interplay of hormonal and other factors, including mechanical ones, necessary for ovulation is unknown. The ovary is brought to ovulation by the action of a pituitary gonadotropic hormone or hormones. Besides causing follicular growth and antra formation, they produce a marked vascular hyperemia, and changes in the ovarian connective tissue stroma which may favor follicle growth and ovulation (29). Follicle and thecal elements are transformed into a corpus luteum through the action of the luteinizing hormone, and it is inferred that the secretion of progesterone begins promptly, if indeed it has not already begun. There are numerous indications that progesterone, presumably secreted by granulosa cells, may be intimately involved in the process of ovulation itself (6, 80, 139, 153). The continued functioning of the corpus luteum has been ascribed to stimulation by a third member of the gonadotropic complex which may be identical with prolactin (9, 62, 134, but cf. reference 50).

Estrogens continue to be secreted throughout gestation in all species studied. In the opinion of Witschi (199), with which the present author is disposed to agree, ovarian estrogen is a secretion of granulosa and luteal cells. Androgens are also normally present in the female and may increase during pregnancy. They may be considered in part a product of the ovarian medulla (146), in view of Hill's experiments (91) on the androgenic potentiality of the ovaries.

a. Endocrine Role of Estrogen. An ovarian estrogenic hormone is dominant at the time of fertilization, and it is upon structures prepared by estrogen that progesterone acts. Estrogen produces cellular mitosis and growth (hyperplasia) of the epithelial cells and glandular structures of the genital tract (1); water uptake (7); synthesis of actomyosin, the contractile protein of uterine smooth muscle (46); glycogen deposition in the circular and longitudinal muscle cells of the uterus (19), and in the muscle cells of vessels; changes in epithelial alkaline phosphatase (10); disaggregation of connective tissue ground substance and increase in water soluble mucoprotein (135). The estrogen-stimulated uterus

takes up vital dyes, a phenomenon interpreted as indicating increased vascular permeability (86) or ground substance change (71). Endometrial ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and succinoxidase are also increased (183). A critical review (181) examines the role of estrogens and other steroids in uterine metabolism.

b. Endocrine Role of Progesterone. Progesterone imposes a qualitative change in the development of the uterus prepared by estrogen, a change already perfectly appreciated by Marshall in 1910 (127). An enormous complication, the progestational proliferation, occurs in the glandular structure (93). The uterus is less hyperemic than after estrogen; glands are dilated and deeply inserted into the submucosa and may be coiled, as in the ewe (15, 40). Cells of the mucosa are more columnar, and mitoses, if any, are rare. The progestational reaction in the rat is accompanied by an increase in endometrial carbonic anhydrase (117) of unknown significance. Progesterone may modify the action of estrogen on the myometrium by altering the permeability to potassium of the muscle cell membrane (46).

The inhibition of estrus and ovulation in pregnancy is usually attributed to progesterone, acting to suppress pituitary hormones responsible for follicular growth. This undoubtedly is a simplification of a still obscure process involving both local and humoral factors. Certainly a role of estrogen, well known to inhibit the production and output of pituitary gonadotropin, may be entertained.

c. Nidation, Pseudopregnancy. The normal effective stimulus to the preparation of an implantation site is the blastocyst, which lies free in the uterus for a variable time, from a few days in rodents to upward of 40 days in the cow and mare, and then attaches at the future placental site. Growth of the blastocyst depends on the presence of progesterone (44). Progestational proliferation provides increased uterine surface and increased glandular activity, and may direct implantation through chemotactic secretions arising from epithelial cells under the influence of progesterone (18).

False, or pseudopregnancy is a normal phase of the cycle of the dog and marsupial cat, where the corpus luteum of the cycle persists and becomes actively functional. In other forms, mechanical stimulation of the genitalia, electroshock, drugs, or sterile mating may artificially induce ovulation and corpus luteum formation. Pseudopregnancy results in the adoption of the habitus of pregnancy in the dog and rabbit, and is normally terminated by breakdown of the proliferated uterus with some bleeding. It may be induced in castrated females by the use of estrogen and progesterone in optimal proportions and amounts (47). Progester-

one by itself is ineffective, while excess estrogen inhibits proliferation. When, concurrently with natural or induced pseudopregnancy, sterile threads are placed in the uterus, active proliferative and inflammatory reactions at the traumatized site are obtained. The placentomata thus formed present a model of pregnancy in the absence of the fetus. Destruction of the fetus at some point in gestation, with maintenance of the true placenta for varying lengths of time (141), gives another model of pregnancy.

d. Endocrine Function of Relaxin. The water-soluble, polypeptide hormone, relaxin, isolated from ovarian extracts by Hisaw (92), has the property of relaxing the symphysis pubis of the guinea pig and other burrowing animals. The effect is maximal just before parturition. In other animals, it relaxes either the symphysis pubis (man, monkey) or other joints related to a widening of the birth canal, e.g., the sacroiliac joints in cow and sheep (68, 95). In addition, relaxin induces an increase in water uptake of the uterus, vagina, and cervix, and relaxation of the cervix in cow (76), sow, and man (202). Relaxin appears to be synthesized in increased amounts in pregnancy, and extraovarian sources have been postulated (202).

Relaxin affects connective tissue, and actions both on the fibrillar (182) and nonfibrillar elements have been postulated. In the latter view, softening and hydration of the symphysis pubis is related to disaggregation of glycoprotein colloids of the matrix, with uptake of water and electrolyte (152). Relaxin normally acts on tissues prepared with estrogen and progesterone, and estrogen itself induces retrogression of fibrocartilage while potentiating the action of relaxin (182).

e. Maintenance of Pregnancy. The earlier literature (see reference, 145, pp. 196-197) indicated that ovariectomy at almost any time terminated pregnancy, by abortion or resorption, in rabbit, rat, mouse, guinea pig, dog, goat, and cow. Later work has shown the ovary to be dispensable in man after the 40-60th day (189), in the mare after the 200th of a 350-day pregnancy (83), and in the ewe after the 55th day of a 150-day pregnancy (138). The termination of pregnancy by hypophysectomy is usually attributed to removal of hormones stimulating the formation of estrogen and progesterone by the ovary. Pregnancy may be maintained in ovariectomized animals by the injection of estrogen and progesterone in the proper amounts and proportions varying with the species (47). Reduction of the fetuses to one in the rat, leaving the placental sites intact, permitted pregnancy to continue after castration (84). In the cat, fetuses were resorbed but the placentas were maintained after oophorectomy, and uterine hypertrophy was retained (47). Variations

on these themes may generally be interpreted by supposing that the placenta produces both estrogen and progesterone, which alone, or in conjunction with administered hormones, may be able to sustain pregnancy or "limited" pregnancy.

It is timely to inquire into the necessity of estrogens for pregnancy, for even where the ovary is dispensable, alternative sources have invariably been found. Fetal death following oöphorectomy could be partially circumvented (67) by slitting the uterus and releasing the fetus into the intraperitoneal cavity. Selye *et al.* (168) attributed death to a lack of uterine resilience. Expressing this view in different but related terms, it is suggested that the function of estrogen in pregnancy is a modification of the uterine ground substance associated with increases in the water-soluble mucoprotein and mucopolysaccharide moieties. The resultant increase in plasticity (70) would conduce to growth and adaptation of uterine muscle cells and connective tissue (collagen) fibers. The hormone relaxin may play a role in this process, and placental estrogens may be visualized as acting either locally or systemically.

2. *The Pituitary Gland of Pregnancy*

Early work is reviewed in the invaluable publications of Van Dyke (187) appearing in the middle thirties. Pituitary enlargement in pregnancy was remarked upon in the human by Comte (43) and by Erdheim and Stumme (60), and occurs in the mouse, cat, and macaque. There was no correlation, however, between fetal length and maternal pituitary weight in the cow (137) or in the mare, as quoted from the same paper. Cytological work was dominated by the concept of a specific "pregnancy cell" (60) variously considered to be a modified oxyphil, chromophobe, or even basophil cell. Innumerable studies on all forms of animals have left this problem in a largely static condition, and the fact remains that the traditional avenues of investigation of the pituitary gland by tinctorial methods, with few exceptions, do not contribute to a knowledge of hormone localization, production, storage, or output. The use of histochemical methods for a specific hormone or hormone complex, as exemplified by localization of the gonadotropic hormones in basophils (26, 88, 150) does not seem to have been routinely applied to the hypophysis of pregnancy. The application of methods based on antigen-antibody localization (128) have not been realized for any hormone of the pituitary other than ACTH. Using another technique of possible application, Herlant (89) found prolactin in an acidophilic granule fraction separated from sheep pituitaries by differential centrifugation.

a. *Biological Actions.* Biological assays of pituitary glands of preg-

nancy for their content of gonadotropin, while somewhat contradictory, have shown on the whole a falling content with the passage of gestation. Van Dyke reviewed the earlier work, which was frequently based on inadequate controls and methods. Smith (173) summarized European findings that the pituitary of pregnant women has a low activity after the 2nd month. Activity in the rabbit was very low after mating, but increased until mid-pregnancy and then fell (90). Assays of individual glands, using two test methods, indicated a gradual and significant fall in activity in the pregnant cow (Table I) (137). A qualitative impression was obtained (31) that activity in the mare hypophysis

TABLE I
GONADOTROPIC ACTION AND POTENCY OF THE PITUITARIES^a OF PREGNANT COWS (137)

Length of fetus (cm.)	Duration of preg- nancy (days)	Rat ovary weight ^b (mg.)	Rat ovary weight ^c (mg.)	Chick testis weight ^d (mg.)
0.1-5.0	< 61	33	74	56
5.1-10.0	61-77	28	70	46
10.1-20.0	78-108	26	70	43
20.1-32.0	109-140	27	61	46
32.1-50.0	141-177	25	57	37
50.1-70.0	178-222	21	42	34
70.0	222	20	36	36

^a Acetone-dried powders of individual pituitary glands were assayed.

^b Received 25 mg. powder/test animal.

^c Received 50 mg. powder/test animal.

^d Received 25 mg. powder/test animal.

similarly falls off, but interpretation was complicated by the possibility of high-titer blood contamination in early pregnancy. Ladman and Runner (112), by an ingenious assay method, attempted to answer, in mice, the difficult questions of hormone synthesis and storage *versus* hormone release. They attribute a low activity in late pregnancy to a phase of rapid release of hormone with no synthesis. Their results, which question the view that high glandular content parallels output, have been critically examined (50). The argument that the low activity of the pituitary in late pregnancy may denote increased release of gonadotropin has never been particularly compelling, and the steady rise in circulating estrogen would point to a suppression of secretion (137). The problem of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) content of pituitaries in pregnancy is one that could be attacked by a serious study of slaughterhouse material, using hypophysectomized assay animals (cf. reference 171).

Assays for the third member of the gonadotropin complex, prolactin

or luteotropin, have scarcely been attempted (8), and the earlier determinations were understandably directed to the postpartum period. Available evidence shows increases through parturition in rat, guinea pig, and rabbit, and increased amounts in both pregnancy and parturition in goat and cow. However, it is fair to add that there are negative reports (64).

For ACTH, thyroid stimulating hormone (TSH), and somatotrophic hormone (STH) no systematic pregnancy studies are available. That "growth hormone" may be released during pregnancy appears probable from the results of hypophysectomy: the total weight of fetuses and placentas was significantly decreased in rats hypophysectomized on the 12th day (109), while monkey fetuses that went to term in hypophysectomized mothers were generally smaller than the norms (174).

b Role in Maintenance of Ovary and of Pregnancy Pregnant animals subjected to hypophysectomy appear to tolerate the operation in inverse proportion to their dependence on the ovary as a source of estrogen and progesterone. Thus, again, is related to the time at which the operation is performed. Intolerant of hypophysectomy at any time are the rabbit (194) and ferret (122). Dogs may be operated upon in late pregnancy but not at 5 and 7 weeks (5), guinea pigs at 40-41 days but not at 34-36 days (151). In the rat, hypophysectomy not more than 4 days after coitus prevented implantation, at 7-10 days there was fetal death and resorption, and after the 11th day, fetal death or persistence and possible prolongation of pregnancy, with the birth of dead or living young. Monkeys (*Macaca mulatta*), in the remarkable series of Smith (174), came to term 20-131 days after hypophysectomy between the 27th and 156th days after conception.

Conclusion Experience with the common laboratory animals shows that fetal death and resorption are universal if hypophysectomy is performed before mid-pregnancy, varying degrees of failure are encountered thereafter, although animals can and do go to term. The primate (monkey) is a distinct exception. Successful replacement therapy in animals, both gonadectomized and hypophysectomized (119), leaves little doubt that hypophysectomy removes the stimulus to corpus luteum maintenance and progesterone production. The contribution of ovarian estrogen is probably impaired also.

c Relation to Other Endocrine Glands of the Mother Removal of the hypophysis during pregnancy led to decreased adrenal weights in mice (69), but to little difference in rats (77). In the monkey, in spite of some lipid loss in cells, the adrenals were protected against the expected weight loss, indicating some extrapituitary source of ACTH.

in the placenta, fetal hypophysis, or both (175). Both in rat and monkey, involution of the thyroid followed hypophysectomy, in pregnant as in nonpregnant animals.

3. *Other Endocrine Glands in Pregnancy*

In the intact animal, homeostatic adjustments may occur during pregnancy in many or all endocrine glands. These changes are often poorly understood and attempts to analyze them by traditional procedures have not always succeeded. Reports that an animal does as well without a known endocrine gland as with it may reflect an ignorance of the external or internal adjustments that have been made. Pregnancy involves energy and material shifts into which endocrine control may enter, both as an essential stimulus and as a means of securing an optimal result.

a. Thyroid. The thyroid hypertrophies in pregnancy, corresponding to increases in the height of follicle epithelium cells and follicle size. Basal metabolic rate and protein-bound serum iodine tend to rise (47, 165). Thyroidectomized rats maintained pregnancy (111) and suckled young (140). The latter observation is disputed, for Folley (63) found an impairment of lactation that was only partially alleviated by giving parathyroid extract. Goats thyroidectomized in pregnancy developed udders normally, but milk production was subnormal (176). It is well known that subsequent phases of lactation in the cow are definitely stimulated by thyroxine or by iodinated proteins (17, 65).

b. Parathyroids. Evidence for increased parathyroid activity during pregnancy is based on gland enlargement, a parathyroid-like activity of the serum of pregnancy, and the relative sensitivity of the pregnant animal to parathyroidectomy (20). Cows thyro-parathyroidectomized in late pregnancy went to term. There was a marked decrease in serum calcium, and a smaller one in inorganic phosphate; it was suggested that the normal bovine diet with its high Ca/P ratio was able to maintain these animals in pregnancy, parturition, and early lactation (179). Milk production was subnormal. The diffuse distribution of the parathyroid glands in the cow may make complete parathyroidectomy difficult or impossible (Boda, unpublished). Goats fared somewhat worse: tetany developed before, though not after, parturition; udder development failed and lactation was nil (176).

c. Adrenal Glands. The literature, as reviewed in a critical and experimental paper by Davis and Plotz (52), reveals complex interrelations between the maternal (and fetal) adrenals and the placenta and the ovaries, based in part on the putative secretion by all these structures

of steroid hormones having parallel or reciprocal actions. Thus, progesterone in large doses maintains adrenalectomized animals fairly well, and the secretion of endogenous progesterone may explain the ameliorating effect of pregnancy on adrenalectomy. On the other hand, the adrenal gland itself synthesizes progesterone (12) which may supplement ovarian or placental lack.

Performed under ideal conditions at least, adrenalectomy is consistent with pregnancy in man (52) and the dog (47). The adverse effect of adrenalectomy on lactation may well be related to its role in maintaining fluid balance, rather than to a direct mammogenic action.

In spite of conflicting reports for laboratory rodents, Davis and Plotz saw no evidence for simple hypertrophy of the adrenal in cat, dog, sow, cow, and ewe. In the rat, they found an absolute increase in the ratio of capillary surface to tissue volume in the zonae fasciculata and reticularis, but not in the glomerulosa or medulla. This increase may be related to the general finding of increased corticosteroid hormone excretion in pregnancy.

d. Posterior Pituitary Gland. There is evidence that the hormones oxytocin and vasopressin, associated with the posterior pituitary, are neurohormones secreted by cells of the hypothalamus (167). During the major part of pregnancy, the normal sensitivity of the uterine smooth muscle to oxytocin is suppressed by progesterone action, ensuring a contraction-free uterus. There seem to be no studies, serial or otherwise, of the oxytocin content of the posterior pituitary and adjacent stalk and brain areas in pregnancy. The other main posterior pituitary function, which has been essentially identified with oxytocin, and to a lesser degree with the vasopressin polypeptide, is the ejection of milk (65), with which we are not here directly concerned.

4. Hormone Levels in Pregnancy

Increased or decreased amounts of hormone secreted by a given gland may show up as a corresponding change in hormone content of the blood and other body fluids: bile, milk, saliva, and, where the hormone molecule can pass the kidney filter, the urine. Readjustments of pregnancy have been seen to result in increased activity in several glands, and with the rise of the placenta as an active organ of secretion, increases in hormone levels in pregnancy are a common finding.

To be considered in this section are the steroid hormones: estrogens, androgens, progestins, and adrenal cortical steroids; the gonadotropins, including prolactin; relaxin and oxytocin. The literature of some of these was extensively documented by Cowie (49) when he surveyed the field

of pregnancy diagnosis by hormonal means in man and the domestic animals. Since this publication is an irreplaceable source of information, it will be freely quoted in order to avoid duplication of references.

a. *Steroid Hormones.* Extensive assays of the biologically active steroids succeeded the discovery by Zondek (203) of increased estrogens in the urine of pregnant women. Latterly, with the advent of chromatographic and other methods for separating, and adsorption methods for identifying active steroids and their inactive metabolites, a complex science has been erected dealing with biosynthesis, interconversion, and metabolism of these products (57). These methods have scarcely been applied to pregnancy in any systematic way.

TABLE II
STEROIDS OF THE URINE OF PREGNANT WOMEN
A. *Metabolites Related to Estrogens (57)*

Phenolic	
Ketonic	Non-ketonic
Estrone ^a	Estradiol-17 β ^a
16 α -Hydroxyestrone ^b	Estriol ^a
16 β -Hydroxyestrone	Estriol glucuronide
	16-Epiestriol

^a Active estrogens.

^b Possible intermediate between estrone and estriol (128a).

B. *Metabolites Related to Progesterone, Androgens, and to the Adrenal Cortical Steroids (57)*

Non-phenolic	
Ketonic	Non-ketonic
Pregnan-3 α -ol-20-one	Pregnane-3 α ,20 α -diol ("pregnenediol")
Allopregnan-3 α -ol-20-one	Allopregnane-3 α ,20 α -diol
Allopregnan-3 β -ol-20-one	Allopregnane-3 β ,20 α -diol
Allopregnan-3 α ,6 α -diol-20-one	Pregnan-3 α -ol
Androsterone	
Dehydroisoandrosterone	

(i) *Estrogens.* The primary estrogen synthesized in the ovary and possibly the placenta of most mammals is estradiol-17 β . This occurs in reversible equilibrium with estrone, and both are converted irreversibly to estriol. The isomeric estradiol-17 α has been found in the mare and goat. Other active and some inactive phenolic steroids basically similar to estradiol are present in the urine of different species. A compilation is given for the pregnant woman (Table IIA), mare (Table IIIA), cow (Table IVA), and goat (Table V), showing estrogen derivatives

TABLE III
STEROIDS OF THE URINE OF PREGNANT MARES
A *Metabolites Related to Estrogens (147)*

Phenolic		
Ketonic	Non-ketonic	Non-phenolic
Estrone (sulfate)	Estradiol-17 β	Δ^5 7 β -Estratrienol-3-one-17
Equilin	Estradiol-17 α	3-Deoxyequilenin
Hippulin	β -dihydroequilenin	
Equilenin		

B *Metabolites Related to Progesterone, Androgens, and to the Adrenal Cortical Steroids (57, 147)*

Non-phenolic	
Ketonic	Non ketonic
Allopregnan-3 β -ol-20-one	Pregnane-3 α ,20 α -diol ("pregnandiol")
Pregnane-3,20-dione	Allopregnane-3 β ,20 α -diol
Allopregnane-3,20-dione	Allopregnane-3 α ,20 α -diol
Δ^6 -Allopregnen-3 β -ol-20-one	Allopregnane-3 β ,20 β -diol
Epiandrosterone	Allopregnane-3 α ,16 α ,20 α -triol
Dehydroisoandrosterone	Urene-3 β ,11-diol
Androstan-3 β -ol-16-one	Urene-3 α ,11,20-triol
Uran-11-ol-3-one	Δ^5 -Pregnene-3 β ,20 α -diol
	17-Methyl-D-homoandrostane-3 β ,17 α -diol
	5 α -Androstane-3 β ,16 β -diol

TABLE IV
STEROIDS IN THE URINE OF PREGNANT COWS
A *Metabolites Related to Estrogens (107, 157)*

Phenolic	
Ketonic	Non-ketonic
Estrone ^a	Estradiol-17 α ^a
	Equol (isoflavon-7,4-diol) ^b

^a Active estrogens

^b Probably dietary

B *Metabolites Related to Progesterone, Androgens, and to the Adrenal Cortical Steroids (50, 117, 148)*

Non-phenolic	
Ketonic	Non-ketonic
Androsterone	Allopregnane-3 α ,20 α -diol
Dehydroisoandrosterone	Allopregnane-3 β ,20 α -diol ("pregnandiol" absent) ^c
	5 β -Androstane-3 α ,17 α -diol
	5 α -Androstane-3 β ,17 α -diol

^c Markert *et al.* (125) found pregnandiol, now thought to be a fecal contaminant

isolated and identified; all of these doubtless represent quantitatively greater amounts than are found in the corresponding nonpregnant forms. Some of them, e.g., equilin and related compounds in the mare are specific to this species, and some may be specific to the state of pregnancy also.

In the urine, the phenolic steroids are present largely as conjugates with glucuronic or sulfuric acid, the biological activity of these being considerably less than that of the parent compounds, although recoverable by hydrolysis. In the blood, estrogens are largely conjugated as water-soluble glucuronides, or as estroproteins (180). Earlier indications that a changing ratio of combined to free estrogen might have an importance in initiating parturition have not been satisfactorily elucidated.

TABLE V
STEROIDS OF THE URINE OF PREGNANT GOATS
Metabolites Related to Estrogens and Androgens (107)

Phenolic		
Ketonic	Non-ketonic	Non-phenolic
Estrone ^a	Estradiol-17 α ^a Equol (isoflavon-7,4-diol) ^b	5 α -Androstane-3 α ,17 α -diol

^a Active estrogens.

^b Probably dietary.

For our purposes, the more significant findings relate to estrogen titers throughout pregnancy. In man, there is a consistent rise to a maximum of 20,000 $\mu\text{g./l.}$ urine, with an abrupt fall at parturition. In the mare, hormone appears in the urine at the 54th day, increases to maximum values between 200 and 300 days, then falls somewhat before term (41), with a precipitous fall at birth (Table VI). The Kober and Cuboni tests for pregnancy in the mare depend on the fluorescence of the phenolic estrogenic steroids with warm sulfuric acid (see reference 49, pp. 81-83). The ass is said to excrete estrogens and it would not be surprising to find them in large amounts in pregnant zebras, kiangs, and mixed equine pregnancies. Mare serum becomes estrogen-positive at the 80th day and increases its titer throughout pregnancy in parallel with the urine.

Estrogens are present in low amounts in pregnant cow serum. The isolation of estrone and estradiol from the bile of pregnant cows (Table VIIA) confirms the secretion of these steroids in enhanced amounts, and also the existence of an enterohepatic circulation of estrogens (149). The urine of pregnant cows has been extensively studied (Table VIII), but the reports are mostly old. There is a rise from the beginning of the

second trimester until term (94, 144), while estrone has been isolated from late pregnancy urine (157). The urine of pregnant sheep, pigs, and goats contains estrogens or their metabolites, and the estrogen of sheep urine rises in pregnancy (Table IX) (195). Estrogen in pregnant pig urine is stated to be present from the 23rd to the 31st day, then to disappear until the 12th week, when it increases until term (see reference 49, pp. 90-91). These early findings are worthy of confirmation and extension. Blood and urine studies on the smaller laboratory animals have been negative or noncontributory (see reference 49, pp. 93-94).

TABLE VI
ESTROGENS IN THE URINE OF PREGNANT THOROUGHBRED MARES (41)

Duration of pregnancy (days)	Rat units of estrogens per liter of urine		
	Mare C6	Mare C8	Mare C3
0- 49			
50- 74	< 125	261*	
75- 99	< 125	< 521-521	
100-124	2,083-4,167	1,041	333-667
125-149	4,167	4,167	1,667
150-174	8,333-16,667	8,333	1,667-3,333
175-199	8,333	8,333	5,000
200-224	16,667	8,333-16,667	5,000-33,333
225-249	8,333-16,667	16,667	8,333
250-274	8,333	16,667-4,167	
275-299	8,333-4,167	8,333	16,667-8,333
300-324	4,167-1,014	4,107	8,333-4,167
325-349	2,083-261	6,333-2,083	8,333-4,167
Postpartum			
2	< 200	< 132	< 84

* At day 54, the earliest date on which estrogens were found.

Conclusion: Estrogens are formed in increasing amounts during pregnancy in many, and perhaps all, mammals, arising partly in the ovary, but later principally in the placenta. They may be detected in blood, urine, and many other maternal and fetal secretions.

(ii) **Progesterone.** Progesterone does not appear in the urine of man, as shown by failure to recover it from enormous volumes of urine (126); animal urine is also negative. A biologically inactive metabolite, pregnanediol, is excreted as the glycuronide in increasing amounts with the progress of gestation in man. Compounds having affinities with progesterone are excreted in the urine of man (Table IIB), mare (Table IIB), cow (Table IVB), and sow (Table X); they may usually be recognized by the sign "pregnan" or "pregnen" in the chemical name of the compound. Both the proportions and kinds of progesterone metab-

TABLE VII
STEROIDS IN THE MILK OF PREGNANT COWS (149)
A. *Metabolites Related to Estrogen*

STERIODS IN THE BILE						
A. <i>Metabolites Related to Estrogen</i>						
Batch bile	Volume (liters)	Assay (untreated) (M.U.)	Free phenols (M.U.)		Strongly acidic	Conjugated phenols (after hydrolysis) (M.U.)
			Weakly acidic			
			Ketonic ^a	Non-ketonic ^b		
I	8.0	64,000	33,000	12,000	1,000	6,600
II	23.0	620,000	120,000	54,000	33,000	64,000

^a Major estrogen: estrone.

^b Major estrogen: estradiol-17 β .

B. *Metabolites Related to Progesterone and to Adrenal Cortical Steroids (57)*

Non-phenolic	
Ketonic	
Pregnan-3 α -ol-20-one	
Non-ketonic	
Pregnane-3 α -20 β -diol	

olites differ from species to species; the sow, for example, excreting none of the common metabolites of man, cow, or mare, while neither the cow nor the ewe excretes pregnanediol.

TABLE VIII
ESTROGENS IN THE URINE OF 9 PREGNANT COWS (94)

Duration of pregnancy (days)	Estrogen (R.U./24 hours)
31	0
44	41
47	0
128	307
154	266
167	960
188	186
214	215
254	667
260	3250
261	483
270	4773
280	6636
280 (term)	3240

TABLE IX
ESTROGENS IN THE URINE OF 2 PREGNANT EWES (195)

Duration of pregnancy (days)	Estrogeos (total) (I.U./24 hours)
46	30?
63	81?
115	120
122	121
123	400
141	> 400

TABLE X
STEROIDS IN THE URINE OF PREGNANT SOWS
Metabolites Related to Progesterone and to the Adrenal Cortical Steroids (57, 147)

Non-phenolic	
Ketonic	Non-ketonic
Pregnan-3 α -ol-20-one	
Allopregnan-3 β -ol-20-one	

The technical problem of estimating progesterone in blood was solved by Hooker and Forbes (97), who used microinjection of serum into the uteri of ovariectomized mice, with hypertrophy of stromal nuclei as the end point. The test may also measure certain progesterone metabolites

and results are expressed as progesterone-equivalents. In the pregnant ewe (Table XI) and rabbit, titers rose progressively and were still high at term (138). Results in man and mouse were less easy to interpret. Progesterone was identified chemically after isolation from systemic blood and adrenal venous blood of both nonpregnant and pregnant cow and ewe, but the data were insufficient to relate to the physiological state (Section IIA.4.a.iii).

TABLE XI
PROGESTIN IN THE BLOOD OF NONPREGNANT AND PREGNANT EWES (138)

Reproductive state	No. animals	Duration (days)	Progesterin-equivalent ^a (μg./ml.)
Estrous cycle	3	1 (estrus)	1.0
	3	8-12 (luteal phase)	4.0-6.0
Pregnancy	5	20	4.0
	5	40	6.0
	5	60	6.0
	5	80	6.0
	5	100	7.0
	5	120	9.0
	5	140	9.0 (8.0-12.0)
Postpartum	4	1.5	6.0
	4	10	2.0

^a Method of Hooker and Forbes (97).

Conclusions: In all intensively studied animals, the urine of pregnancy contains metabolites of progesterone in amounts that increase with the progress of gestation. The paths of metabolism are not the same for all species, and systematic study of a given form should include a knowledge of the range of possible metabolites. Progesterone was present in the blood of all species tested and may be estimated chemically and biologically.

(iii) *Adrenal cortical steroids.* Steroids recognized as proper to the adrenal cortex include two groups of hormones primarily affecting carbohydrate and salt metabolism, respectively. Compounds of the first group of which corticosterone is representative, possess an oxygen atom at the C₁₁ position and were originally isolated from the adrenal gland as "cortin." Active hormones or metabolites of this class appear in the urine and have been assayed biologically and shown to increase in pregnancy (188). The prototype of the second class is the synthetic hormone deoxycorticosterone, lacking oxygen at C₁₁. However, the principal naturally occurring representative is aldosterone (electrocortin). Increasing amounts of this hormone are excreted in the urine after the 3rd

month of human pregnancy and remain elevated till term (190). Extension of this observation to other forms has not been made, nor has its significance been appraised.

Progesterone has been identified as an intermediate in the biosynthesis of adrenal cortical steroids (87). It is present in the adrenal venous blood of cattle, sheep, and pigs in amounts 10–100 times its concentration in arterial blood (12). Consequently, metabolites of ovarian and placental progesterone will be mingled with those of adrenal progesterone and related adrenal steroids in the common urinary pathway, and are listed jointly (Tables IIB, IIIB, IVB, VIIB, and X). It would be a general inference that substances arising via the adrenal synthetic route are increased in pregnancy.

Estrogens are produced by certain adrenal tumors, and their metabolism follows, in part, familiar, in part, unfamiliar routes (147). It is not known if a significant amount of estrogen is normally produced by the adrenal gland, or if it varies in pregnancy.

Androgens in the female are believed to be largely products of the adrenal cortex, and their metabolic contribution is to the non-phenolic 17-ketosteroids of the urine. Some of these have been found in pregnant human, mare, cow, and goat urine (Tables IIB, IIIB, IVB, and V) (they carry the chemical sign "androstan" or "androsten"). The evidence that they increase in pregnancy is equivocal. A biologically active androgen found to increase in the pregnant monkey (58) appears to have a placental origin, and, of course, a placental source is very likely for a part of the heterogeneous group of metabolites related to progesterone.

b. Gonadotropic Hormones. (i) *Chorionic gonadotropin (man); equine gonadotropin (mare).* Chorionic gonadotropin is found in the urine (3, 4) and blood of pregnant women. It produces in the ovaries of immature test animals (mice and rats): follicular ripening and ovulation, hyperemia, hemorrhage into follicles (blood points), and luteinization of both ruptured and unruptured follicles. Secondly, estrogen secretion produces a cornified vaginal smear, and uterine hyperemia and growth. More chronically, the effects of heavy luteinization become prominent. These effects form the basis of pregnancy diagnosis tests in man and have evoked an immense literature (see reference 49, p. 23 ff.). Serial determinations of gonadotropins in human pregnancy were made by Evans *et al.* (61), and by Browne and Venning (24). Highest amounts, up to 1,000,000 I.U./l. were present from the 6th to the 10th week, falling rapidly thereafter to some 10,000 I.U./l. which value was maintained till term. Peak blood levels followed a parallel course with maximum values of 70,000 to 600,000 I.U./l. between the 50th and 65th day.

In the pregnant mare, gonadotropic hormone appears only infrequently or in traces in urine; equine gonadotropin, when injected, disappeared slowly from the blood of rabbits and of a gelding (28), whereas chorionic gonadotropin is rapidly cleared from blood. Equine gonadotropin is present in the blood of pregnant mares over a relatively limited span of gestation (38). Adequate assays have been provided over extended periods for different breeds of horses by Cole and Saunders (41) for thoroughbreds (Tables XII), by Day and Rowlands (54) for ponies of various breeds, and for Welsh ponies (Table XIII) by Cole (36) and

TABLE XII
GONADOTROPINS IN THE BLOOD OF PREGNANT THOROUGHBRED MARES^a (41)

Duration of pregnancy (days)	Rat units ^b of gonadotropins per liter of blood			
	Mare C6	Mare C8	Mare C3	Mare C4
43	60	100		
45	500	300		
47		1,562		
49	6,250	3,125		6,250
50-59	12,500-50,000	25,000-50,000		25,000
60-69	50,000	50,000		12,000
70-79	50,000	50,000		6,250
80-89	50,000-25,000	50,000		1,562
90-99	50,000-25,000	50,000	25,000	1,562
100-109	25,000	50,000	12,500	780
110-119	12,500-6,250	25,000-2,500	6,250	400-200
120-139	3,125-1,562	12,500-6,250	3,125-1,562	100
140-159	780-390	3,125-1,625	781-400	

^a Includes the mares of Table VI, with one addition.

^b This rat unit is equivalent to 1 I.U. equine gonadotropin.

TABLE XIII
GONADOTROPINS IN THE BLOOD OF PREGNANT WELSH PONIES (36)

Duration of pregnancy (days)	Animal No.									
	1	2	3	4	5	6	7	8	9	10
	All values in rat units = 1 U./ml.									
55-59		100			25		100		200	200
60-64	100 ^a	100	200	200-400		100		100	200	200
65-69	100		400				100			
70-74	100, 200 ^a	200, 50 ^a		400	100	100	100			
75-79		100 ^a	266							
80-84								100		

^a These values were obtained during a second year of testing the same animals.

by Aylward and Ottaway (11) Maximal values of 50,000 I U /l were found for the larger breeds and up to 400,000 I U for ponies between the 55th and 110th day of gestation, it is well documented that the smaller pony breeds have the highest titers. Hormone falls off rapidly from peak values, titers usually have definitely fallen by the 110th day, and by the 150th day hormone is for all intents and purposes absent. The question of the source of equine gonadotropin is considered later (Section III A 2). Equine gonadotropin, present in the blood serum of pregnant mares from a relatively early date, provides an effective means of pregnancy diagnosis, and has been widely exploited (see reference 49, pp 50 55).

(u) *Gonadotropins in other animals* With the exception of the primates macaque, chimpanzee, and orangutan (see reference 49, p 67), gonadotropic hormones are absent from the blood and urine of other animals, including ewe, goat, sow, bitch. For the pregnant cow, assiduous attempts to develop a biological pregnancy test leave little doubt that active gonadotropin is absent (see reference 49, pp 62 66, but cf reference 48).

(iii) *Prolactin (luteotropin)* Assuming the identity of luteotropin with prolactin, the hormone was reported in postpartum urine (120) and in the urine of newborn infants (118), where it provides the basis of "witches milk." Otherwise, its distribution in pregnancy has not been systematically studied, until this is done, many features of its dual action must remain obscure.

c *Relaxin* Increased amounts of this hormone appear in the blood of the pregnant sow, cat, mare, rabbit, man, and guinea pig (95, 202). Titers were low in early pregnancy, increased to plateaus in the last trimester, and fell rapidly after delivery. There was good correlation of hormone level with its physiological action of symphyseal loosening in the final stages of pregnancy in the guinea pig (30).

d *Oxytocin* Progesterone decreases the sensitivity of the uterus to oxytocin, while estrogen increases it. Parturition could be initiated by a rising estrogen or falling progesterone level in the blood, or by a rising estrogen/progesterone ratio, or by an increased secretion of oxytocin. None of these has been unequivocally shown to occur, and concepts of the mechanism of parturition, which have not changed much in 50 years (163), fall back vaguely on some combination of these processes as the endocrine component of birth.

5 Growth and Involution of the Uterus

Bulk growth of the uterus in pregnancy is achieved by the coordination of three processes at least, independently of circulatory changes

There is a massive hypertrophy, without hyperplasia, of uterine muscle cells, with synthesis of actomyosin and other components of the contractile system (46); an extensive synthesis of collagen (81); and a striking increase in the extracellular, interfibrillar ground substance (124). Collagen increase is shown by the extraction of a water-insoluble, gelatinizable protein fraction determined as hydroxyproline. Ground substance, by virtue of its content of mucoprotein and mucopolysaccharide, is demonstrated histologically by the periodic acid leucofuchsin reagent. These changes are conditioned, and can be in part reproduced, by estrogen. Comparable, if less sweeping, changes occur in the sterile horn of a bicornuate uterus. The presence of the conceptus introduces a mechanical or stretch factor which may provide one element of the stimulus for protein synthesis and growth (81, 162). Biochemical stimuli must also arise from the presence of the blastocyst, if one considers, for example, the highly specific changes that occur in the fertile uterine horn of the pregnant mare (Section III.A.2.). The process of uterine involution has been unaccountably neglected, and is shrouded perhaps in rather more mystery even than uterine growth. Its initial stages, at least, are exceedingly rapid: there is a rapid and progressive loss of collagen (82), of muscle cell cytoplasm, and of ground substance (124). In the clinical and veterinary literature, the process is described almost exclusively in terms of pathological alteration: fatty degeneration, cell lysis, hyaline change, and the like. It may be of interest to record that none of these common "degenerative" changes were observed in the involuting uterus of the rat (124).

6. *The Mammary Glands in Pregnancy*

The animal enters its first pregnancy with a mammary gland that has been subjected to hormonal stimulation characteristic of the preceding estrous cycles; there will be distinct species differences relative to this prior experience. The rabbit, with no spontaneous ovulation, shows a system of ramifying ducts, with rudimentary alveoli; the bitch, with its prolonged and functional luteal phase, shows elaborate alveolar development, simulating true pregnancy. Following mammary growth, lactation, and involution of pregnancy, the mammary gland will retain increasing amounts of both parenchymal and connective tissues, and in successive pregnancies will start from a modified base line. Mammary gland growth is dominated by steroid hormones proceeding from the ovary or alternative sources. As a first approximation, mammary development involves three stages: the formation of a more or less elaborate duct system, conditioned by estrogen; the addition to this of the lobular-alveolar system,

conditioned by progesterone; the initiation and maintenance of lactation, a function of prolactin. Qualitatively, these effects may overlap. Thus, estrogens alone produce extensive duct and alveolar development, with supervening lactation, in the guinea pig, goat, and cow (51). Progesterone alone, in high doses, promotes both duct and alveolar growth in several species (64). Anterior pituitary extracts induce mammary hyperplasia in the castrate rabbit (45). More refined experimental analysis, e.g., the factorial design adopted by Benson *et al.* (16), emphasizes optimal hormonal environment for lactogenesis and presents the following progesterone-estrone ratios as favorable for full mammary development: guinea pig, 100:1-20:1; rabbit, 40:1-10:1; rat, 150:1-90:1; goat, 140:1. While adequate figures are woefully lacking, it may be of interest to give a rough computation for the cow. Taking blood estradiol values in the pregnant cow (180) and converting to estrone equivalent by the factor of Reece (160) and using the progesterone value of Neher and Zarrow (138) for the luteal phase, the progesterone-estrone value works out at 190:1-50:1, figures of the same order of magnitude as those quoted above.

The major part of mammary growth in pregnancy occurs during the first half or two thirds (184) of the total span, and may be attributed to ovarian estrogen and progesterone in the earlier phases and to placental augmentation later. From the work of Benson *et al.* (16), it would be anticipated that a favorable ratio would be maintained throughout, but how this is effected is unknown. In late pregnancy, there is a glandular swelling resulting from the accumulation of a watery or milky secretion. Descriptions of glandular growth in pregnancy have been given for various rodents (184) and for the cow (79).

B. Placental Endocrine Functions

Newton (142), in his article on hormones and the placenta, gave a masterly summary of the position to that time, and a critique which it would be hard, even at this time, to equal. He believed that the placenta had an endocrine function in the control of gestation, but that this was difficult to express in precise terms, or to prove in detail. The placenta, like the liver, is a massive metabolizing, as well as transmitting, organ and has been revealed as a store of practically everything that has been sought in it. Newton showed the placenta further to be an autonomous organ which, independently of the fetus, was able to maintain the aspect of pregnancy, and which itself has a precisely delimited life *in utero*. Techniques of great promise, which have been added since the above, are those of placental perfusion and the use of tagged molecules.

1. Estrogens

Lipoid extracts causing vaginal cornification were early obtained from the placentas of man, cow, sheep, and pig (reference 145, Table 8). The isolation of estriol and its water-soluble glucuronide from human placenta was an important theoretical advance (42). Use of current chromatographic methods should continue to reveal a wide spectrum of steroid hormones and related substances in placentas; by these means, estrone and estriol were isolated from cow placenta (155). Perfusion of human placenta seems to have answered in the affirmative whether steroids are synthesized there, although estrogens have not thus far been identified (155). The basic biological arguments *pro* and *con* placental synthesis were considered by Newton. Possibly the continued excretion of estrogens following double oöphorectomy in man and mare provide the best evidence. In man, the number of cases is small, but circumstantially conclusive (189). In the mare, the issue is complicated by the presence of enlarged and seemingly active fetal gonads (Section III.A.3). But, for several reasons, this is an unlikely source of estrogens, especially in late pregnancy; on the strength of Hart and Cole's single case (83) one would be prepared to accept placental synthesis of estrogen in the mare without the reservation made by Newton in favor of the fetal gonads.

2. Progesterone

Progesterone was early detected in human placenta (see reference 142, p. 426). When the corpus luteum of pregnancy was removed at 40, 54, and 98 days in man (189), the excretion of pregnanediol (and estrogen) continued to increase, if not to the same degree as normally. Perfusion of human placenta showed steroidogenesis of two substances closely related to progesterone, though not of progesterone itself, while addition of radioactive (C_3^{14}) progesterone to the perfusate gave these same two substances, which are evidently metabolites of progesterone (78). The excretion of pregnanediol, which is still high at term, ceases abruptly at parturition. Kimura and Lyons (105) did not find progesterone (biologically) in chorion or endometrium of the mare, but Short (170) by chemical methods found several hundred micrograms/kilo. However, using the same techniques adequate for man and mare, he found none in cow, ewe, sow, or bitch placentas. Assuming that metabolites will eventually be detected, there seems to be every reason for accepting progesterone synthesis by the placenta.

3. Adrenal Cortical Steroids

Perfusion of human term placentas with whole blood led to the appearance of steroid substances in the chromatographic positions of

deoxycorticosterone, cortisol, and tetrahydrocortisol (155). The last-named group was increased in amount by the addition of ACTH to the perfusate.

4. ACTH, Thyrotropin

As already mentioned, the results of hypophysectomy in the macaque indicate that the placenta may secrete ACTH but not thyrotropin (175). There are conflicting reports on ACTH in rat placenta (77, 100) and a comparative extension of these findings would be hazardous.

5. Relaxin

The rabbit placenta contains considerable amounts of relaxin (202). Placentas of other forms have not been studied.

6. Gonadotropins

Gonadotropic activity was found in implanted human placenta at 7 and 11 weeks, and at term (204). The fetal placenta of the pig was negative (154). Hormone was present in extracts of allantochorion and fertile endometrium of the pregnant mare, in amounts that roughly paralleled the rise and fall of the hormone in the blood stream (31). The allantochorionic fluid was negative, as were extracts of the endometrium of the infertile horn.

Implant (104) and tissue culture (73, 99) experiments placed the locus of formation of the human gonadotropin in cells of the chorionic villi. The source of equine gonadotropin was originally considered to be the fetal placenta (31). Later, Cole and Goss (37) adduced evidence that specialized "endometrial cups" of the fertile endometrium were responsible (Section III.A.2).

Gonadotropic hormones have been sought in the placentas of most domestic and laboratory species. Many of these observations are not particularly recent. Stated to be negative for gonadotropic activity are: placenta, endometrium, and amniotic fluid of the cow and sheep; placenta of the sow, rabbit, and guinea pig (see reference 49, pp. 62-67).

a. Chemistry and Biology of the Gonadotropins of Pregnancy. The gonadotropins of pregnancy are produced in relation to the placenta of man (chorionic gonadotropin) and to the fertile endometrium of the mare (equine gonadotropin). Their function in these species, as well as their apparent absence in others, presents an unsolved puzzle. Both substances resemble the gonadotropic hormones FSH and LH isolated from the anterior pituitary gland in containing considerable amounts of hexose and hexosamine in their molecules: they are glycoprotein hormones. Nevertheless, they differ in chemical details and in

biological properties from the pituitary hormones and from each other (115). Equine gonadotropin possesses a full range of gonadotropic activity comparable with that of unfractionated pituitary extracts (34); it is biologically equivalent to FSH plus LH but, on the other hand, has not been successfully resolved into separate components. Chorionic gonadotropin, while active in the intact animal, is limited in effect in the hypophysectomized one, and has been considered to be largely LH-like.

A reconstruction of the functions of these hormones must be hypothetical. One purely endocrine view would refer to the massive luteinization produced in the ovary of the pregnant mare around the 60th day of pregnancy, indicating a prolongation of the secretion of progesterone as a main function. In man, luteinization appears not to be induced in the ovaries of pregnancy, which in any case are shortly dispensable. However, the addition of a sheep pituitary gonadotropin to the perfusate of human placenta apparently increased progesterone synthesis (78). This preparation contained at least FSH and LH and the experiment demonstrates the possibility of a local action in the placenta of a pituitary complex.

Another view, not mutually exclusive to the above, would point to certain ancillary actions of gonadotropic hormones on the connective tissue and blood vascular systems of the gonad (29). It was also suggested that mesenchymal cells of the fetal placenta could be the target of chorionic (and equine) gonadotropin (71). In a quite remarkable passage, Marshall (127) referred to the "synthesis of intercellular glycoprotein mucin by the ovum." Possibly the function of these hormones may lie somewhere in the formation (and role) of the placental glycoproteins.

C. Endocrine Functions of the Fetus

In intrauterine life, the primordia of the endocrine glands are laid down and become differentiated, and by the time of delivery can usually be shown to possess a histology of adult type and to contain detectable amounts of their characteristic hormones. A systematic study would seek to determine, among other facts: (1) the acquisition of competence of the fetal gland to produce secretions of recognizable type, whether "adult" or "fetal" in nature; (2) the interaction of fetal endocrine systems with each other; (3) the response of developing systems to maternal hormones; (4) the contribution of the fetal secretions to the total complex (132). Questions (3) and (4) include the problem of placental transmission of the various hormones. Needless to say, few of these ob-

jectives have been satisfactorily achieved for any single fetal endocrine gland.

1. *Fetal Gonads.*

Surgical or X-ray castration of fetuses rather late in intrauterine life has resulted in partial or complete failure in development of the male (Wolffian duct) accessories, and the persistence of female (Müllerian duct) structures (102, 159, 193). These abnormalities were prevented by implantation of testosterone propionate, supporting the view that testicular androgen is produced before birth and serves to maintain fetal accessories.

Implants of fetal rat testes gave androgenic stimulation of adult seminal vesicles (101), while extracts of embryonic bull testes (201) furnished evidence of androgen content, if not necessarily of synthesis. The presence of male sex hormone in fetal horse gonads was not definitely shown (39). The suggestion that sex hormones control differentiation of the sex cells was made by Bouin and Ancel (22) and became the basis of the freemartin hypothesis (103, 116). In bovine twinning, with fusion of the chorioallantoic vessels, a genetic female becomes modified in the male direction by sex hormones proceeding from the male co-twin. The ovary fails to develop, and in its place a testis forms; Müllerian structures degenerate, and Wolffian elements persist and develop.

The acquisition of an ability of the fetal gonads to respond to hormones normally present in the maternal-fetal milieu has been studied by means of experiments which have had as their objective the deliberate modification of gonads and sex tracts by male and female sex hormones. There are many competent reviews of this extensive and highly complex subject (25, 133, 199, 205). A capacity to react to hormones of the adult type is shown by differentiated structures, primordia, and even anlagen of the fetal genital tract, to give more or less far-reaching modifications of the Müllerian and Wolffian ducts, the urogenital sinus, and the external genitalia. In the mammal at least, efforts to obtain a definitive gonadal reversal, as in the freemartin, have not succeeded. The later writings of Moore (131), who was closely associated with Lillie, struck a pessimistic note: doubting that the freemartin is susceptible of a hormonal explanation, and negating the role of fetal gonadal hormones. A cautious attitude would regard this pessimism as not entirely justified if the few positive indications be weighed against the many negative ones, with the following provisos in mind: whether a continuous, inexorable secretion of minute amounts of hormone into the fetal circulation has often been realized experimentally; whether, except

rarely, the critical stages of development have been so exposed. These are characteristically the conditions that obtain in pregnancy.

2. The Fetal Thyroid

The thyroid gland acquires the facility of accumulating iodine at a definite point in fetal life. This occurs near term in the mouse and rat (75, 178); at mid-pregnancy in the pig (158); at the beginning of the second trimester in man (32, 96) and sheep (13); and around the end of the first quarter in the bovine (110, 200). The initial finding of iodine may coincide with follicle formation (mouse, rat, sheep) or be unrelated to follicles or to colloid (pig, cattle), indicating that synthetic potency does not depend on cell disposition in the tissue. However, in later intrauterine life the accumulation of large amounts of iodine is invariably linked with follicle growth and colloid formation. The fetal sheep, which begins to accumulate radioiodine by the 50th day (Table XIV), can synthesize iodothyronine, triiodotyrosine, and thyroxine by the 70th day (75a).

TABLE XIV
INITIATION OF THYROID FUNCTION IN FETAL SHEEP (13)

Duration of gestation (days)	Per cent of maternal dose of I^{131} in fetal thyroid	Histological notes
46	0	Cordlike epithelial cells, no follicles
48	0	Cordlike epithelial cells, no follicles
50	0.0001	Cordlike epithelial cells, no follicles
52	0.04	Few small distinct follicles
58	0.70	Limited number of follicles, in periphery
60	0.28	Limited number of follicles, in periphery
70	0.44	Many colloid-filled follicles throughout gland
75 ^a		
145 (term)		

^a Iodothyronine, triiodotyrosine, and thyroxine are being synthesized by this date (75a).

The fetal thyroid of the rat responds to the direct injection of TSH at the 19th day. Hypophysectomy of the fetus did not produce a distinct effect on the histology of the fetal thyroid, but the elapsed time was short (169).

3. The Fetal Adrenal

The fetal adrenal in several species is characterized by a thin outer cortical zone, which becomes the definitive cortex, and a thick inner zone the androgenic, X-zone or provisional zone (52, 198). For this reason the fetal adrenal is large relative to body weight at birth. After birth

the X-zone involutes and the gland becomes absolutely and relatively smaller in size. Although biologically active androgen was not found in fetal human glands (72), newborn infants excreted relatively large amounts of 17-ketosteroids in the first 2 days of life; thereafter amounts fell rapidly (161). Davis *et al.* (53), using tagged C^{14} -acetate, found a high rate of cholesterol synthesis in fetal adrenals (human) of the 3rd to 5th month of intrauterine life; this would agree with the finding of glucocorticoids in the newborn (191).

The fetal hypophyseal-adrenal system appears to be active before birth (106). Decapitation of fetuses (hypophysectomy) reduced the volume of the adrenal glands; ACTH repaired the loss and stimulated the adrenals even further. It has long been known that adrenalectomy of the mother results in fetal adrenal hypertrophy (98); this was recently confirmed by adrenalectomy at the 14th day of pregnancy (108). Since the effect is abolished by hypophysectomy, it is postulated that an increase of maternal ACTH, which crosses the placenta, is responsible.

III. SPECIAL ASPECTS OF PREGNANCY IN DOMESTIC ANIMALS

A. Mare (Tables IIIA, IIIB, VI, XII, XIII, XV)

1. Equine Gonadotropin and the Ovaries

The primary corpus luteum of pregnancy is short-lived. It fills the cavity of the ruptured follicle by the 4th-6th day, reaches maximal size by the 14th day, and begins to regress at the end of the first month. Suppression of follicular activity is incomplete, and ovulation has been observed on the 23rd day. Following the appearance of gonadotropin in the blood at the 40th-50th day, there is intense ovarian stimulation, ovulation, and formation of a new crop of corpora lutea. Tubal ova were recovered from mares in foal examined at 46-73 days (2); their fertilizability was not established, but the absence of superfetation in the mare speaks against it. The newly formed corpora are in regression by the 150th day, when gonadotropic hormone has largely disappeared from the blood.

2. The Equine Placenta

Placental attachment, simple though it is, becomes better defined after the 40th day. Until that time, the blastodermic vesicle falls away from the opened uterus under its own weight, although the site is marked by an area of endometrial hyperemia. Coincidentally, at this point, highly specialized, villate, endometrial outgrowths, the "endometrial cups," are thrown up, closely invested by the allantochorion (37). The uterine glands in the cups enlarge and fill with a mucoprotein secretion

rich in gonadotropic hormone, which has been assayed at successive stages of pregnancy (Table XV). Endometrial eup activity, and in fact the activity of the fertile horn generally, closely parallels that of the blood. The superficial eup secretion collects in allantochorionic sacs that tend to become pedunculated (37, 55). Clegg *et al.* (32a) propose an origin for the hormone in epithelial and glandular cells of the eup, although a portion of the histochemically demonstrable material would appear to be mucin. These workers observed also faintly staining mucoprotein granules in undifferentiated uterine mesenchymal cells and in decidual cells presumably derived from them, but considered them to be unrelated to the hormone. The present author, having been once bitten on this issue, is disposed to leave open the question of the cellular origin of the hormone. In passing, it may be remarked that by definitely relegating gonadotropin secretion to the endometrium, we are left without a reasonable function for the remarkable, hypertrophied, apocrine glandlike cells of the chorion which are prominent at this time (31).

TABLE XV
GONADOTROPIC HORMONE ACTIVITY OF "ENDOMETRIAL CUPS" AND CUP SECRETION
IN PREGNANT MARES^a (32a)

Crown-rump length of fetus (cm.)	Approximate duration of pregnancy (days)	No. animals	Average weight of cups and secretions (g.)	Total gonadotropic activity (I.U.)	Serum activity (I.U./ml.)
2.0-4.9	40 (2.0 cm.)	12	3.5	21,000	0.1-6.0
5.0-9.9		14	8.0	56,000	11-160
10.0-14.9	80 (10.0 cm.)	11	5.5	73,000	6-296
15.0-19.9	105 (19.7 cm.)	4	10.5	150,000	< 1-93
20.0-24.9		9	8.6	133,000	< 0.5-106
25.0-29.9	150 (25-30 cm.)	10	5.2	42,000	
30.0-34.9	180 (34 cm.)	3	2.9	14,000	
35.0-45.0					< 0.1-0.2

^a Range mustangs, originating in western United States (39).

3. The Fetal Gonads

Beginning at a fetal crown-rump length of 20 cm. (approximately 100 days), the fetal gonads of both sexes undergo a massive proliferation of interstitial cells. The gonads reach a maximum size at 45-60 cm. (6½-8 months), when the pair may weigh 50-100 g., then diminish to about one fifth of this at term. In the process of growth, the sex cells are restricted to a narrow cortical zone in the female and to scattered cords in the male (39).

The stimulus for interstitial cell growth in the fetal gonads was left unsettled by the above authors, though some writers have assumed it to be maternal gonadotropin. While the effect accords with that of a gonadotropin, the fetal gonads reach their maximum size some 2 months after gonadotropin has disappeared from the blood, and long after the maternal ovaries have regressed, this temporal dissociation is difficult to explain. There is a much closer correlation with maternal estrogen, which has been considered by some to be the effective stimulus (2). But, for the time being, it seems best to leave this question open.

The fetal gonads themselves contain estrogen and possibly male sex hormone, but no progesterone. It was originally thought that they might contribute to the high titers of estrogen in late pregnancy, but since fetal blood, liver, kidney, placenta, and gonads proved to be essentially equivalent in estrogen content, the idea was discarded (27).

B Cow (Tables I, IVA, IVB, VIIA, VIIB, VIII)

1 Cervical Mucus

The secretion of cervical mucus is under the control of the ovarian hormones. Estrogen produces a clear, thin mucus, progesterone a tougher secretion, the flow properties of mucus represent the resultant of the action of these hormones on the glands of the anterior vagina and cervix. A composite flow parameter, θ , which measures the hardening and softening of a mucus sample under a shear stress, was determined (74). Data from 40 pregnant cows indicated that in 50% of animals there was a significant increase in θ (i.e., increased tackiness) at 3½ weeks, and in 95% after 8 weeks.

2 Nidation

Details of the attachment of the bovine ovum have been provided by the study of 18 heifers and cows killed 676–927 hours after ovulation (130). The uterine caruncles are present in fetal life and do not fluctuate with the cycle. Their response to the trophoblast is a loss of surface epithelium, which later extends to the rest of the uterine surface. By the 800th hour, a fragile attachment at 2 or 4 caruncles surrounding the fetus has been made, by 850 hours, interdigitation has progressed so that the embryo is being nourished by the cotyledons. The hormonal stimulus to caruncular growth would seem to be progesterone acting in an estrogen primed environment, but direct evidence is not available.

3 Corpora Lutea and Maintenance of Pregnancy

Ablation of corpora lutea in 10 cows between the 92nd and 236th days caused prompt abortion. Pregnancy was maintained in 8 out of 9

cows with corpora ablated at the 60th day, but receiving a daily intra-muscular injection of 100 mg. crystalline progesterone. In such animals, discontinuance of the treatment at the 162nd day did not produce abortion (121). The inference seems to be that endogenous progesterone and estrogen are produced and that the pregnancy becomes "stabilized" following ovarian loss and treatment.

4. Progesterone and Ovulation

Histochemical studies of uterine glycogen and alkaline phosphatase, although their significance is unknown, suggested that progesterone is secreted at fairly high levels both before and after estrus (136). There have been several confirmations of the somewhat paradoxical finding that progesterone may potentiate ovulation in cattle (Section II.A.1) and other forms.

5. Relaxin and the Cervix Uteri

Using a special mechanical dilator, dilatation of the cervix was only possible during estrus. After sensitization with 20 mg./day stilbestrol, the subcutaneous injection of relaxin provoked cervical relaxation to the following degrees: 200 guinea pigs units (G.P.U.), dilatation 0.9 inches; 1500 G.P.U., dilatation 1.27 inches; 8500 G.P.U., dilatation 1.34 inches. The median dose was considered optimal (76).

C. Goat (Table V)

1. Maintenance of Pregnancy

Goats were laparotomized on the 100th or 125th day of pregnancy, and corpora lutea shelled out of the ovary. With all corpora removed in 4 animals and double oophorectomy in 1, abortion occurred 2 days later, confirming the necessity of progesterone in the last trimester. In 20 out of 21 goats, the number of corpora equaled the number of fetuses. When one-half the corpora were excised, 3 goats, each bearing 2 fetuses, continued in pregnancy for varying periods. With all corpora removed, 25 mg. daily of progesterone injected subcutaneously maintained pregnancy in 3 out of 3 goats; 15 mg. daily, in 4 out of 4 goats; and 10 mg. daily, in 4 out of 6 goats. This roughly establishes the 50% survival dose, and the rate of endogenous progesterone synthesis, for the pregnant goat (129).

D. Sow (Table X)

1. Ovarian Relaxin

The relaxin content of the pregnant sow ovary increased from 500 G.P.U./g. fresh tissue with a $\frac{3}{4}$ inches fetus, to 10,000 G.P.U./g. fresh tissue with a 5 inches fetus, as compared with values of 2.5 to 5 G.P.U./g

in the luteal phase of the cycle and less than 1 G.P.U./g. in the follicular phase. The placenta at fetal stages from 4½ to 10 inches contained 0.5–2.5 G.P.U./g., with no correlation with fetal size. Although there are indications of extragonadal sites of origin of relaxin, the corpus luteum of pregnancy appears to be the main site of synthesis (95, 202).

E. Ewe (Tables IX, XIV)

1. Estrus in Pregnancy

Estrous behavior appears to be common in the pregnant ewe. Periods of heat at intervals of 3–40 days, with a mean of 22 days, were not restricted to any one part of the gestation period and were not accompanied by ovulation (197).

2. Maintenance of Pregnancy

Abortion follows ovariectomy in the first trimester. Ewes ovariectomized at 84 hours after breeding were maintained in gestation by daily treatment with 1 mg. progesterone and 0.25 mg. estrone (ratio 4000:1), but not by 1 mg. progesterone and estradiol in the ratio of 100:1 (66).

3. Steroid Excretion

Whitten's study was supported by further serial determinations of ketonic (estrone) and non-ketonic (estradiol) fractions in urine and feces of merino sheep. All fractions increased in late pregnancy (14).

4. Milk Ejection

In a rather surprising report, relaxin contracted the mammary alveoli in sheep, although not as completely as did oxytocin (166).

5. Blood Progesterone

Progesterone was determined by the Hooker-Forbes technique. Blood values rose progressively during pregnancy and were still high at term, although they fell sharply a few hours after birth. Ovariectomy at the 66th and 114th day did not interrupt pregnancy, nor materially change the time course of blood progesterone values (138).

REFERENCES

1. Allen, E., in "Sex and Internal Secretions" (E. Allen, ed.), p. 392. Williams & Wilkins, Baltimore, Maryland, 1932.
2. Amoroso, E. C., and Rowlands, I. W., *J. Endocrinol.* 7, Proc. 1 (1951).
3. Aschheim, S., *Arch. Gynäkol.* 132, 179 (1927).
4. Aschheim, S., and Zondek, B., *Klin. Wochschr.* 6, 1322 (1927).
5. Aschner, B., *Arch. ges. Physiol., Pflüger's* 146, 1 (1912).
6. Asdell, S. A., in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 3, Chapter 16. Butterworths, London, 1957.

7. Astwood, E. B., *Endocrinology* **23**, 25 (1938).
8. Astwood, E. B., *Ciba Colloq. Endocrinol.* **5**, 74 (1950).
9. Astwood, E. B., and Fevold, H. L., *Am. J. Physiol.* **127**, 192 (1939).
10. Atkinson, W. B., and Elftman, H., *Endocrinology* **40**, 30 (1947).
11. Aylward, F., and Ottaway, C. W., *J. Comp. Pathol. Therap.* **55**, 159 (1945).
12. Balfour, W. T., Comline, R. S., and Short, R. V., *Nature* **180**, 1481 (1957).
13. Barnes, C. M., Warner, D. E., Marks, S., and Bustad, L. K., *Endocrinology* **60**, 325 (1957).
14. Beck, A. B., *Australian J. Agr. Research* **1**, 322 (1950).
15. Bell, T. D., Casida, L. E., and Darlow, A. E., *Endocrinology* **28**, 441 (1941).
16. Benson, C. K., Cowie, A. T., Cox, C. P., and Goldzweig, S. A., *J. Endocrinol.* **15**, 126 (1957).
17. Blaxter, K. L., *J. Endocrinol.* **4**, 237, 266 (1945).
18. Bloch, S., *J. Endocrinol.* **1**, 399 (1939).
19. Bo, W. J., and Atkinson, W. B., *Anat. Record* **113**, 91 (1952).
20. Bodansky, M., and Duff, V. B., *J. Am. Med. Assoc.* **112**, 223 (1939).
21. Böving, B. G., *Am. J. Anat.* **98**, 403 (1956).
22. Bouin, P., and Ancel, P., *Compt. rend. soc. biol.* **55**, 1682 (1903).
23. Brambell, F. W. R., *Proc. Roy. Soc. B* **130**, 462 (1942).
24. Browne, J. S. L., and Venning, E. M. H., *Lancet* **ii**, 1507 (1936).
25. Burns, R. K., in "Survey of Biological Progress" (G. S. Avery, ed.), Vol. 1, p. 233. Academic Press, New York, 1949.
26. Catchpole, H. R., *J. Endocrinol.* **6**, 218 (1949).
27. Catchpole, H. R., and Cole, H. H., *Anat. Record* **59**, 335 (1934).
28. Catchpole, H. R., Cole, H. H., and Pearson, P. B., *Am. J. Physiol.* **112**, 21 (1935).
29. Catchpole, H. R., Gersh, I., and Pan, S. C., *J. Endocrinol.* **6**, 277 (1950).
30. Catchpole, H. R., Joseph, N. R., and Engel, M. B., *J. Endocrinol.* **8**, 371 (1952).
31. Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.* **55**, 167 (1934).
32. Chapman, E. M., Corner, G. W., Robinson, D., and Evans, R. D., *J. Clin. Endocrinol.* **8**, 717 (1948).
- 32a. Clegg, M. T., Boda, J. M., and Cole, H. H., *Endocrinology* **54**, 448 (1954).
33. Cohen, S. L., Marrian, C. F., and Watson, M., *Lancet* **i**, 674 (1935).
34. Cole, H. H., *Am. J. Anat.* **59**, 299 (1936).
35. Cole, H. H., *Am. J. Physiol.* **119**, 704 (1937).
36. Cole, H. H., *Proc. Soc. Exptl. Biol. Med.* **33**, 193 (1938).
37. Cole, H. H., and Goss, H., in "Essays in Biology in Honor of Herbert M. Evans," p. 107. Univ. of California Press, Berkeley, California, 1943.
38. Cole, H. H., and Hart, C. H., *Am. J. Physiol.* **93**, 57 (1930).
39. Cole, H. H., Hart, C. H., Lyons, W. R., and Catchpole, H. R., *Anat. Record* **66**, 275 (1933).
40. Cole, H. H., and Miller, R. F., *Am. J. Anat.* **57**, 39 (1935).
41. Cole, H. H., and Saunders, F. J., *Endocrinology* **19**, 199 (1935).
42. Collip, J. B., Browne, J. S. L., and Thomson, D. L., *J. Biol. Chem.* **97**, xvii (1932).
43. Comte, L., *Beitr. pathol. Anat. u. allgem. Pathol.* **23**, 90 (1898).
44. Corner, C. W., *Am. J. Physiol.* **86**, 74 (1928).
45. Corner, G. W., *Am. J. Physiol.* **95**, 43 (1930).
46. Corner, C. W., and Csapo, A., *Brit. Med. J.* **i**, 687 (1953).

- 47 Courier, R, "Endocrinologie de la gestation" Masson, Paris, 1945
- 48 Cowie, A T, *Vet Record* 63, 371 (1951)
- 49 Cowie, A T, *Commonwealth Agr Bur (Gt Brit) Joint Publ No 13* (1948)
- 50 Cowie, A T, and Folley, S J, in "The Hormones" (G Pincus and K V Thumann, eds), Vol 3, p 309 Academic Press, New York, 1955
- 51 Cowie, A T, Folley, S J, Milpress, F H, and Richardson, K C, *J Endocrinol* 8, 64 (1952)
- 52 Davis, M E, and Plotz, E J, *Obstet Gynecol Survey* 11, 1 (1956)
- 53 Davis, M E, Plotz, E J, LeRoy, G V, Gould, R G, and Werbin, H, *Am J Obstet Gynecol* 72, 740 (1956)
- 54 Day, F T, and Rowlands, I W, *J Endocrinol* 2, 255 (1940)
- 55 Day, F T, and Rowlands I W, *J Endocrinol* 5, 1 (1947)
- 56 Dorfman, R I, in "The Hormones" (G Pincus and K V Thumann, eds), Vol 1, p 467 Academic Press, New York, 1948
- 57 Dorfman, R I, and Ungar, F, "Metabolism of Steroid Hormones" Burgess, Minneapolis, Minnesota, 1953
- 58 Dorfman, R I, and van Wagenen, C, *Surg Gynecol Obstet* 73, 545 (1941)
- 59 Dutt, R H, *J Animol Sci* 13, 464 (1954)
- 60 Erdheim, J, and Stumme, E, *Beitr pathol Anat u ollgem Pathol* 46, 1 (1909)
- 61 Evans, H M, Kohls, C L, and Wonder, D H, *J Am Med Assoc* 108, 287 (1937)
- 62 Evans, H M, Simpson, M E, and Lyons, W R, *Proc Soc Exptl Biol Med* 46, 586 (1941)
- 63 Folley, S J, *J Physiol* 93, 401 (1938)
- 64 Folley, S J, in "Marshall's Physiology of Reproduction" (A S Parkes, ed), Vol 2, p 525 Longmans, Green, London 1952
- 65 Folley, S J, *The Physiology and Biochemistry of Lactation* Oliver & Boyd, Edinburgh and London, 1956
- 66 Foote, W D, Gooch, L D, Pope, A L, and Gasida, L E, *J Animal Sci* 16, 986 (1957)
- 67 Frazer, J D F, *J Physiol* 130, 253 (1955)
- 68 Frieden, E H, and Hisaw, F L, *Recent Progr in Hormone Research* 8, 333 (1953)
- 69 Gardner, W U, and Allen, E, *Annot Record* 83, 75 (1942)
- 70 Gersh I, *Harvey Lectures Ser* 45, 211 (1952)
- 71 Gersh, I, and Catchpole, H R, *Am J Anat* 85, 457 (1949)
- 72 Gersh I, and Grollman, A, *Am J Physiol* 126 368 (1939)
- 73 Gey, C O, Seegar, C E, and Hellman, L M, *Science* 88, 306 (1938)
- 74 Glover, F A, and Scott Blair, G W, *J Endocrinol* 9, 160 (1953)
- 75 Gorbman, A, and Evans, H M, *Endocrinology* 32 113 (1943)
- 75a Gorbman, A, Waterman, A, Barnes, C M, and Bustad, L K, *Endocrinology* 60, 565 (1957)
- 76 Graham E F, and Dracy, A E, *J Dairy Sci* 36 772 (1953)
- 77 Greer, M A, *Endocrinology* 45, 178 (1949)
- 78 Hagopian, M, Pincus, G, Curlo J, and Romanoff, E B, *Endocrinology* 58, 387 (1956)
- 79 Hammond J, "The Physiology of Reproduction in the Cow" Macmillan New York, 1927
- 80 Hansel, W, and Trimmerger, G W, *J Dairy Sci* 35 65 (1952)

81. Harkness, M. L. R., and Harkness, R. D., *J. Physiol.* **132**, 492 (1956).
82. Harkness, M. L. R., Harkness, R. D., and Moralee, B. E., *J. Physiol.* **132**, 502 (1956).
83. Hart, G. H., and Cole, H. H., *Am. J. Physiol.* **109**, 320 (1934).
84. Haterius, H. O., *Am. J. Physiol.* **114**, 399 (1936).
85. Hechter, O., *Vitamins and Hormones* **13**, 245 (1955).
86. Hechter, O., Krohn, L., and Harris, J., *Endocrinology* **29**, 386 (1941).
87. Hechter, O., and Zaffaroni, A., *Recent Progr. in Hormone Research* **6**, 215 (1951).
88. Herlant, M., *Bull. acad. roy. belg. classe sci.* **26**, 588 (1942).
89. Herlant, M., *Ann. endocrinol. (Paris)* **13**, 611 (1952).
90. Hill, R. T., *J. Physiol.* **83**, 129 (1934/5).
91. Hill, R. T., *Endocrinology* **21**, 495 (1937).
92. Hisaw, F. L., *Proc. Soc. Exptl. Biol. Med.* **23**, 66 (1928).
93. Hisaw, F. L., in "Sex and Internal Secretions" (E. Allen, ed.), p. 499. Williams & Wilkins, Baltimore, Maryland, 1932.
94. Hisaw, F. L., and Meyer, R. K., *Proc. Soc. Exptl. Biol. Med.* **26**, 586 (1928-29).
95. Hisaw, F. L., and Zarrow, M. X., *Vitamins and Hormones* **6**, 151 (1950).
96. Hodges, R. E., Evans, T. C., Bradbury, J. T., and Keettel, W. G., *J. Clin. Endocrinol. and Metabolism* **15**, 661 (1955).
97. Hooker, C. W., and Forbes, T. R., *Endocrinology* **41**, 158 (1947).
98. Ingle, D. J., and Fisher, G. T., *Proc. Soc. Exptl. Biol. Med.* **39**, 149 (1938).
99. Jones, G. E. S., Gey, G. O., and Gey, M. K., *Bull. Johns Hopkins Hosp.* **72**, 26 (1943).
100. Josimovich, J. B., Ladman, A. J., and Deane, H. W., *Endocrinology* **54**, 627 (1954).
101. Jost, A., *Gompt. rend. soc. biol.* **142**, 198 (1948).
102. Jost, A., *Arch. anat. microscop. morphol. exptl.* **39**, 577 (1950).
103. Keller, K., and Tandler, J., *Wien tierarztl. Monatssch.* **3**, 513 (1918).
104. Kido, I., *Zentr. Gynäkol.* **61**, 1551 (1937).
105. Kimura, J., and Lyons, W. R., *Proc. Soc. Exptl. Biol. Med.* **37**, 423 (1937).
106. Kitchell, R. L., and Wells, L. J., *Endocrinology* **50**, 83 (1952).
107. Klyne, W., and Wright, A., *Biochem. J.* **66**, 92 (1957); *J. Endocrinol.* **14**, xxxiii (1956).
108. Knobil, E., and Briggs, F. N., *Endocrinology* **57**, 147 (1955).
109. Knobil, E., and Caton, W. L., *Endocrinology* **53**, 198 (1953).
110. Koneff, A. A., Nichols, C. W., Wolff, J., and Chaikoff, I. L., *Endocrinology* **45**, 242 (1949).
111. Krichesky, B., *Am. J. Physiol.* **126**, 234 (1939).
112. Ladman, A. J., and Runner, M. H., *Endocrinology* **53**, 367 (1953).
113. Lasley, E. L., *J. Animal. Sci.* **16**, 335 (1957).
114. Levine, R., in "Survey of Biological Progress" (B. Glass, ed.), Vol. 3, p. 153. Academic Press, New York, 1957.
115. Li, G. H., *Vitamins and Hormones* **7**, 223 (1949).
116. Lillie, F. R., *J. Exptl. Zool.* **23**, 371 (1917).
117. Lutwak-Mann, C., and Adams, C. E., *J. Endocrinol.* **15**, 43 (1957).
118. Lyons, W. R., *Proc. Soc. Exptl. Biol. Med.* **37**, 207 (1937).
119. Lyons, W. R., *Proc. Soc. Exptl. Biol. Med.* **54**, 65 (1913).
120. Lyons, W. R., and Page, E., *Proc. Soc. Exptl. Biol. Med.* **32**, 1049 (1935).

- 121 McDonald, L E, McNutt, S H, and Nichols, R E, *Am J Vet Research* 14, 539 (1953)
- 122 McPhail, M K, *Proc Roy Soc B* 117, 34 (1935)
- 123 Mahaffey, L W, *Australian Vet J* 26, 267, 295 (1950)
- 124 Maibenco, H C, Ph D Thesis "Postpartum Uterine Involution in the Albino Rat" University of Illinois, Urbana, Illinois, 1957
- 125 Marker, R E, *J Am Chem Soc* 60, 2442 (1938)
- 126 Marker, R E, Kamm, O, and McGrew, R V, *J Am Chem Soc* 59, 616 (1937)
- 126a Marrian, G F, Loke, K H, Watson, E J D, and Panatton, M, *Biochem J* 66, 60 (1957)
- 127 Marshall, F H A, 'The Physiology of Reproduction' Longmans, Green, London, 1910
- 128 Marshall, J M, Jr, *J Exptl Med* 94, 21 (1951)
- 129 Meites, J, Webster, H D, Young, F W, Thorpe, F J, and Hatch, R N, *J Animol Sci* 10, 411 (1951)
- 130 Melton, A A, Berry, R O, and Butler, O D, *J Animol Sci* 10, 993 (1951)
- 131 Moore, C R, 'Embryonic Sex Hormones and Sexual Differentiation' Thomas, Springfield, Illinois, 1947
- 132 Moore, C R, *J Clin Endocrinol and Metabolism* 10, 942 (1950)
- 133 Moore, C R, *J Clin Endocrinol and Metabolism* 13, 330 (1953)
- 134 Moore, W W, and Nalbandov, A V, *J Animal Sci* 12, 950 (1953)
- 135 Moses, L, and Catchpole, H R, *Federation Proc* 14, 104 (1955)
- 136 Moss, S, Sykes, J F, and Wrenn, T R, *Am J Vet Research* 17, 607 (1956)
- 137 Nalbandov, A V, and Casida, L E, *Endocrinology* 27, 559 (1940)
- 138 Neher, G M and Zarrow, M X, *J Endocrinol* 11, 323 (1954)
- 139 Nellor, J E, and Gole, H H, *J Animal Sci* 16, 151 (1957)
- 140 Nelson, W O, and Tobin, G E, *Endocrinology* 21, 670 (1937)
- 141 Newton, W H, *J Physiol* 84, 196 (1935)
- 142 Newton, W H, *Physiol Revs* 18, 419 (1938)
- 143 Newton W H, in 'Marshall's Physiology of Reproduction' (A S Parkes, ed), Vol 2, p 442 Longmans, Green, London, 1952
- 144 Nibler, G W, and Turner, G W, *Proc Soc Exptl Biol Med* 26, 882 (1929)
- 145 Parkes, A S, 'The Internal Secretions of the Ovary' Longmans, Green, London 1929
- 146 Parkes, A S, *Recent Progr in Hormone Research* 5, 101 (1950)
- 147 Pearlman, W H, in "The Hormones" (G Pincus and K V Thimann, eds), Vol 1, pp 351, 407 Academic Press, New York, 1948
- 148 Pearlman, W H, *Biochem J* 67, 1 (1957)
- 149 Pearlman, W H, Rakoff, A E, Cantarow, A, and Paschke, K E, *J Biol Chem* 170, 173 (1947)
- 150 Pearce, A G E, *J Pathol Bacteriol* 61, 195 (1949)
- 151 Pencharz, R E, and Long, J A, *Am J Anat* 53, 117 (1933)
- 152 Perl, E, and Catchpole, H R, *Arch Pathol* 50, 233 (1950)
- 153 Pfeiffer, G A, *Anat Record* 106, 233 (1951)
- 154 Philipp, E, *Zentr Gynakol* 53, 2386 (1929)
- 155 Pincus, G, *Conf on Gestation, Trans 3rd Conf, Princeton, New Jersey, 1956*, p 91 (1957)
- 156 Pomeroy, R W, *Intern Congr on Animal Reproduction, Rept 2nd Conf, Copenhagen, Addendum p 5* (1952)

157. Pope, G. S., McNaughten, M. J., and Jones, H. E. H., *Biochem. J.* 66, 206 (1957).
158. Rankin, R. M., *Anat. Record* 80, 123 (1941).
159. Raynaud, A., *Arch. anat. microscop. morphol. exptl.* 39, 518 (1950).
160. Reece, R. P., *Proc. Soc. Exptl. Biol. Med.* 73, 284 (1950).
161. Reid, C. H., Venning, E. H., and Ripstein, M. P., *J. Clin. Endocrinol.* 10, 845 (1950).
162. Reynolds, S. R. M., "Physiology of the Uterus." Hoeber, New York, 1949.
163. Robinson, T. J., in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 3, Chapter 18. Butterworths, London, 1957.
164. Rowson, L. E., *J. Endocrinol.* 7, 260 (1951).
165. Salter, W. T., in "The Hormones" (G. Pincus and J. V. Thimann, eds.), Vol. 2, p. 181. Academic Press, New York, 1950.
166. Shaffhausen, D. D., Jordan, R. M., and Dracy, A. E., *J. Dairy Sci.* 37, 1173 (1954).
167. Scharrer, E., and Scharrer, B., in "Recent Progress in Hormone Research" (G. Pincus, ed.), Vol. 10, p. 183. Academic Press, New York, 1954.
168. Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology* 19, 151 (1935).
169. Sethre, A. E., and Wells, L. J., *Endocrinology* 49, 369 (1951).
170. Short, R. V., *Nature* 178, 743 (1956).
171. Simpson, M. E., van Wagenen, G., and Carter, F., *Proc. Soc. Exptl. Biol. Med.* 91, 0 (1956).
172. Smith, P. E., and Engel, E. T., *Am. J. Anat.* 40, 159 (1927).
173. Smith, P. E., in "Sex and Internal Secretions" (E. Allen, ed.), p. 931. Williams & Wilkins, Baltimore, Maryland, 1939.
174. Smith, P. E., *Endocrinology* 55, 655 (1954).
175. Smith, P. E., *Endocrinology* 66, 271 (1955).
176. Smith, V. R., Stott, C. H., and Walker, C. W., *J. Animal Sci.* 16, 312 (1957).
177. Spector, W. S., "Handbook of Biological Data." Saunders, Philadelphia, Pennsylvania, 1956.
178. Speert, H., Quimby, E. H., and Werner, S. C., *Surg., Gynecol. Obstet.* 93, 230 (1951).
179. Stott, C. H., and Smith, V. R., *J. Dairy Sci.* 40, 897 (1957).
180. Szego, C. M., and Roberts, S., *Proc. Soc. Exptl. Biol. Med.* 61, 181 (1946).
181. Szego, C. M., and Roberts, S., *Recent Progr. in Hormone Research* 8, 419 (1953).
182. Talmage, R. V., *Anat. Record* 99, 91 (1947).
183. Telfer, M. A., and Hisaw, F. L., Jr., *Acta Endocrinol.* 25, 390 (1957).
184. Turner, C. W., in "Sex and Internal Secretions" (E. Allen, ed.), p. 740. Williams & Wilkins, Baltimore, Maryland, 1939.
185. Umbaugh, R. E., *Am. J. Vet. Research* 10, 295 (1949).
186. Van Demark, N. L., and Salisbury, C. W., *J. Animal Sci.* 9, 307 (1950).
187. Van Dyke, H. B., "The Physiology and Pharmacology of the Pituitary Body," Vols. 1 and 2. Univ. of Chicago Press, Chicago, Illinois, 1935 and 1939.
188. Venning, E. H., *Endocrinol.* 39, 203 (1946).
189. Venning, E. H., *Conf. on Gestation, Trans. 3rd Conf., Princeton, New Jersey, 1956*, p. 71 (1957).
190. Venning, E. H., Primrose, T., Caligaris, L. C. S., and Dyrenfurth, I., *J. Clin. Endocrinol. and Metabolism* 17, 473 (1957).
191. Venning, E. H., Randall, S. P., and György, P., *Endocrinology* 45, 430 (1949).

192. Wells, L. J., *Arch. anat. microscop. morphol. exptl.* 39, 317 (1950).
193. Wells, L. J., Cavanaugh, M. W., and Maxwell, E. L., *Anat. Record* 118, 109 (1954).
194. White, W. E., *Am. J. Physiol.* 102, 505 (1932).
195. Whitten, W. K., *Australian J. Exptl. Biol. Med. Sci.* 21, 187 (1943).
196. Willet, E. L., McShan, W. H., and Meyer, R. K., *Proc. Soc. Exptl. Biol. Med.* 79, 396 (1952).
197. Williams, S. M., Garrigan, V. S., Norton, H. W., and Nalbandov, A. V., *J. Animal Sci.* 15, 978 (1956).
198. Windle, W. F., "Physiology of the Fetus." Saunders, Philadelphia, Pennsylvania, 1940.
199. Witschi, E., *Recent Progr. in Hormone Research* 6, 1 (1951).
200. Wolff, J., Chaikoff, I. L., and Nichols, C. W., *Endocrinology* 44, 510 (1949).
201. Womack, E. B., and Koch, F. C., *Endocrinology* 16, 267 (1932).
202. Zarrow, M. X., *Conf. on Gestation, Trans. 3rd Conf., Princeton, New Jersey, 1956*, p. 17 (1957).
203. Zondek, B., *Klin. Wochschr.* 7, 485 (1928).
204. Zondek, B., *Zentr. Gynäkol.* 55, 1 (1931).
205. Zuckerman, S., *Arch. anat. microscop. morphol. exptl.* 39, 436 (1950).

CHAPTER 15

Factors Affecting Gestation Length and Parturition

M T CLEGG

	<i>Page</i>
I Introduction	509
II Factors Affecting Length of Gestation	509
A Genetic Factors	511
B Environmental Factors	513
1 External Environmental Influences	514
2 Internal Environmental Influences	516
III Prolonged Gestation	519
IV Parturition	522
A Sequence of Events in Normal Labor	523
1 First Stage	523
2 Second Stage	524
3 Third Stage	525
B Biochemical Changes in the Placenta at Term	526
V The Initiation of Parturition	526
A Neural Factors	527
B Endocrine Factors	528
C Physical Factors	533
References	533

I INTRODUCTION

The duration of pregnancy can be defined, in a general sense, as that period which extends from the instant of fertilization of the ovum to the emptying of the uterine contents. Since the exact time of fertilization of the ovum is seldom known, and since pregnancy frequently terminates prematurely as a result of abnormal physiological or pathological conditions, the gestation period as used in this chapter will refer to the length of time from mating to normal parturition.

In Table I, the gestation periods for the common domestic species and breeds are summarized from data compiled by Kenneth (90).

II FACTORS AFFECTING LENGTH OF GESTATION

Factors influencing the length of gestation may be broadly classified into three categories, endocrine, genetic, and environmental. The important role of the endocrines in the maintenance of pregnancy is well known. Since this subject has been thoroughly reviewed in the previous chapter, a further treatment would be repetitious. The present dis-

TABLE I

GESTATION PERIODS OF SOME OF THE COMMON BREEDS OF DOMESTIC LIVESTOCK*

Animal	Average	Minimum	Maximum
Cattle (dairy breed)			
Ayrshire	277.9		
Brown Swiss	289.7	270	306
Friesian	276.3	240	333
Guernsey	283.9		326
Holstein-Friesian	278.8	262	359
Jersey	278.8	270	285
Milking Shorthorn	281.7		322
Swedish-Friesian	281.8	260	300
Zebu	285		
Cattle (beef breed)			
Aberdeen-Angus	279.0		
Hereford	285.1	243	316
Beef Shorthorn	282.9	273	294
Goat			
Anglo-Nubian	149		
Barbari	146		
Bashkir	147.6		
Jamna Pari	150		
Jaaner	153		
Toggenburg	150.5	136	157
Horse			
Arabian	337.0	301	371
Belgian	335.3	304	354
Clydesdale	334.1		
Morgan	343.7	316	363
Percheron	322	321	345
Shire	339.5		
Thoroughbred	338.1	301	349
Pig			
Berkshire	115.1	107	124
Chester White	113.5		
Duroc-Jersey	114.0	102	118
Hampshire	114.1		
Large Black	114.7		
Large White	114.3	103	128
Middle White	113.0		
Norwegian Landrace	114.2	111	119
Poland-China	115.3	113	119
Spotted Poland-China	115.8		
Wild Pig	123.5	124	140

* From data compiled by Kenneth (90).

subsequent investigations have confirmed this observation (4, 14, 82, 83, 123).

From a study of the effect of interbreed difference, intersire difference within breeds, and the association between the time the dam spent *in utero* as compared to her progeny, Brakel *et al.* (17) concluded that genetic factors influenced the duration of pregnancy.

Heritability estimates of gestation length were calculated by Rollins *et al.* (130) from their own data as well as data reported by other workers. These values as quoted from their paper are summarized in Table II. The magnitude of these heritability estimates clearly indicates that the genotype of the fetus is a factor influencing the length of intrauterine fetal life. Gestation length was positively correlated with inbreeding of the dam, but inbreeding of the calf had no effect.

TABLE II
ESTIMATES OF HERITABILITY OF GESTATION LENGTH IN CATTLE

Breed	No. animals in study	Heritability estimate (%)	Comments
Jersey	1000	30	Data adjusted for sex and calving sequence differences prior to estimation of heritability
Holstein	350	32	Same as above
Swedish Friesian	900	43	Calculated as 4x paternal half-sib correlation on basis of material obtained from analysis of variance tables given in author's table
Ayrshire	100 pairs	64	Based on 2x offspring dam correlation given by the authors in their paper
Jersey	100 pairs	36	Same as above

Genetic influences on the gestation length in the horse have not been studied as extensively as in cattle, but the available data indicate that the genotype of the foal exerts an important effect. Rollins and Howell (129) analyzed the recorded gestation lengths of 186 purebred Arabian horses. They found a heritability estimate of 36% after adjusting for the effects of season of breeding and nutrition of the mare. Furthermore, they suggested that sex-linked genes in the genotype of both the fetus and the mare may be of possible importance to the length of gestation.

Information on the influence of the genotype of the lamb on gestation length of domestic sheep is meager. In one excellent study, however, Terrill and Hazel concluded that the hereditary effects exerted by

the lambs and those effects inherent in the ewe accounted for 40 to 50% of the variance of the duration of pregnancy (150). They calculated a heritability estimate of 30 to 40% after adjusting for tangible environmental effects.

Breed differences in the gestation length have also been observed. Medium-wool mutton breeds, such as the Southdown, Hampshire, Shropshire, and Dorset Horn, have a short gestation varying from 144 to 148 days. Fine-wool breeds, such as the Merino and Rambouillet, have a longer gestation, varying from 148 to 152 days. Long-wool mutton breeds, such as the Lincoln and Romney, have periods intermediate in length, varying from 146 to 149 days (150).

The duration of gestation in the goat is similar to that of sheep, ranging from 147 to 155 days. Breed differences also exist; that of the Bar Bari, Angora, and Philippine being shorter than the Jumna Pari, Anglo-Nubian, and Schwartzwald (8).

In the domestic pig, Joubert and Bonsma (85) noted a significant effect of the sire, and Johnson (81) found a small but real difference in the lengths of gestation between breeds. The Poland China averaged almost 3 days longer than the Hampshire.

In beef cattle, the Angus breed has the shortest average gestation period, while Herefords have the longest and Shorthorns are intermediate (23). Although Burris and Blunn (23) were unable to find an effect due to the sire, Gerlaugh (54) reported a significant influence of both the sire and dam on gestation length.

The length of pregnancy in the rabbit varies from 27 to 37 days (Table I) and differs with breed or strain (61). It is generally shortest for the small breeds and longest for the large breeds (159).

From the foregoing, it is clear that the length of gestation is to some extent controlled by hereditary influences, the genotype of the fetus as well as that of the dam being of importance. The nature of their contribution, however, is elusive. Endocrine mechanisms, fetal size, and fetal metabolism have been suggested as possible causes.

B. Environmental Factors

Environmental factors that affect gestation length may be classified as external and internal. The external ones include nutrition and season, which may imply an influence of such things as light and temperature. Fetal size or sex, litter size, and order of pregnancies may be considered as internal factors, and probably exert their ultimate effect by altering the intrauterine environment. In addition, the physiological state of the dam, as influenced by age, health, or weight, may contribute to physiological variations of the uterus.

1. External Environmental Influences

a. *Seasonal.* A seasonal difference in gestation length of cows has been suggested by many studies. In their review, Brakel *et al.* (17) cite many references indicating that those cows freshening in winter had longer pregnancies than those freshening in summer. In many of these analyses, the differences were small and insignificant. In extensive investigations of over 900 gestation periods involving Ayrshire, Holstein-Friesian, and Jersey cows, Brakel *et al.* (17) observed a significant difference in length between spring (March, April, and May) and autumn (September, October, November) calving. Calves born in the spring were carried an average of 2.07 days longer than those born in the autumn months. On the other hand, in studies on a purebred Jersey herd, when differences due to the influence of sire were ruled out, seasonal effects did not contribute to variation in gestation length (130). Similarly, Knapp *et al.* (92) found no differences in length of gestation that could be associated with season in the Shorthorn breed.

Because seasonal influences may depend upon many factors, such as nutrition, sunlight, and exercise, the meaning of a difference associated with season is obscure. The general finding indicates that the month of calving has a slight effect, but probably one of little importance.

In contrast to the cow, the season of breeding of the mare is an important source of variation in gestation length, and, according to Howell and Rollins (75), it accounts for 44% of the total variance. Pregnancies in Arabian mares resulting from breedings during the period extending from December through May were, on the average, 10.4 days longer than those resulting from breeding during the period extending from June through November.

As uterine attachment of the blastocyst is delayed in the horse (25), one may speculate whether seasonal factors influence implantation. External stimuli, such as suckling or light, may control the release of hormones that are known to affect implantation. When lactating mice are bred, nidation of the embryo is delayed and pregnancy is prolonged (91). In the American marten, an excessively long period of time elapses before implantation occurs; this period can be reduced up to three months if the animals are exposed to increased daily light (112).

These observations suggest a neural endocrine mechanism involving the anterior pituitary and its effect upon the secretion of progesterone by the ovary. This hormone is known to influence the time of implantation. For example, delayed nidation can be induced in rats ovariectomized on the third day after mating by the daily injection of

this substance (30). In the author's opinion, studies of factors influencing implantation in the horse may help clarify the way that external environment exerts its effect.

In ewes, the effect of season of breeding upon gestation length appears not to have been studied extensively. In one investigation (150), a trend to longer gestations in ewes bred early in the season was noted. The observed average decrease of 0.03 day in duration of pregnancy for each day of the breeding season was highly significant. As date of breeding contributed only 1% to the total variance, other factors are apparently more important.

In goats, the month of conception has some influence on the length of pregnancy; it averages 151.3 ± 0.1 days for August conceptions and 149.8 ± 0.1 days for February conceptions. It is possible that these differences are associated with nutritional effects either due to insufficiency of available nutrients or because more energy is required to maintain body temperature during the cold winter months (9).

In summary, the effects of season of breeding on gestation length are primarily associated with the nutritional environment to which the animal is exposed during the period of pregnancy. In the horse, however, the influence of the season of mating is largely independent of these factors. "Delayed implantation" may be a major contributing cause accounting for the seasonal differences in length of pregnancy.

b. Nutritional. To the author's knowledge, there have been no studies designed with the specific intent of determining a correlation between inadequate nutrition and gestation length in dairy cattle. These studies would be pertinent in view of the observed nutritional effects in other species and the apparent effect of obesity upon fertility in cows (103).

When mares were maintained on a high nutritional level, pregnancies averaged 4 days shorter than those limited to pasture and oat hay. This difference in level of nutrition was independent of season of breeding and accounted for 5% of the total variance in gestation length (75).

As in other domestic species, studies specifically designed to determine the influence of plane of nutrition on the duration of pregnancy in sheep are limited. The mean gestation period for ewes on a low nutritional intake during the second half of pregnancy was found to be reduced if twins were born. In those sheep bearing single lambs, however, the nutritional level had no influence (151). Recently Alexander (3) placed Merino ewes on high, medium, and low planes of nutrition from the 108th, 129th, 136th, or 143rd day of pregnancy to parturition. The gestation period of animals on a sub-maintenance ration was

shortened from 0.7 to 5.0 days. Furthermore, the heavier the uterine contents at the time the severe undernutritional treatment was commenced, the more parturition was advanced. Thus, the extent of reduction of gestation length induced by undernutrition was greater in older fetuses than in younger, or in twins than in singles.

These results are interesting with regard to the mechanism of parturition. Although this subject will be discussed later, it seems appropriate at the present time to mention the "insufficiency of fetal nutrition theory" suggested by Spiegelberg (145), a concept expressed much earlier by Hippocrates when he wrote: "When the child is grown big and the mother cannot continue to provide him with enough nourishment, he becomes agitated, breaks through the membranes and incontinently passes out into the external world free from any bonds. In the same way among the beasts and savage animals, birth occurs at a time fixed for each species without overshooting it, for necessarily in each nourishment will become inadequate. Those which have the least food for the fetus come quickest to birth and visa versa. And that is all I have to say upon the subject" (122).

Although a theory of parturition based upon fetal nutritional insufficiency is attractive, other effects of undernutrition must also be considered.

Dietary insufficiencies in pregnant animals may involve the endocrine balance. For example, Nelson and Evans (110) showed that pregnancy could be maintained in rats in spite of the absence of dietary protein if the animals were injected daily with progesterone and estrone. Furthermore, these hormone combinations did not cause an increased food intake, a fact which would eliminate nutritional factors as a direct cause.

2. Internal Environmental Influences

The termination of pregnancy implies the end of an intrauterine existence; therefore, any factor influencing gestation must have a final common effect on the uterus. The term "internal environmental influences" as used in this discussion applies to those factors which may have a direct bearing upon the environment *in utero*. Conditions other than hormone concentrations which modify the internal environment include size of fetus, sex of fetus, size of litter, age of dam, and number of pregnancies, any or all of which may play a role in determining the time of parturition.

a. *Number and Size of Fetuses.* Size of the fetus is positively correlated with length of gestation in both dairy and beef animals (17, 18,

23, 92, 130), but the increased weight of the calf is merely the result of a continued growth during the longer period of intrauterine life.

A direct influence of mass of conceptus upon parturition can be better demonstrated if one relates an effect of number of young rather than size of fetus to length of gestation. In sheep, pregnancies terminated by the birth of twin lambs have a shorter duration (0.6 day) than those terminated by the birth of singles (39, 150). When twins are carried in cattle, the length of gestation is reduced 3 to 6 days (4, 14, 94, 161). In goats, although differences in the duration of pregnancy are not significantly associated with number born, there is a tendency for multiple young to be carried for a shorter term (9, 69). Size of litter in pigs does not exert a significant influence on the mean gestation length (85, 95), but, in rabbits, within a breed, the pregnancy is prolonged when the number of young in the litter is small (104). Variation in the duration of pregnancy between breeds, however, is not influenced by litter size, nor is there any evidence, for a breed giving birth to a high average number of young, that the length of pregnancy is necessarily below the average (159).

This conclusion is not incompatible with an influence of conceptual mass upon parturition, since other factors influencing gestation length may be associated with breed differences. Furthermore, the experimental alteration to reduce litter size will prolong pregnancy in rabbits (162).

b. Sex of Fetus. In some species, such as the pig and the rabbit, sex ratio cannot be correlated with length of pregnancy (101). Although, in sheep, the sex of lamb has not always been shown to influence the duration of pregnancy (15, 117), a suggestive increase in the percentage of males with increasing lengths of gestation has been reported (101). Both in cows and horses, the sex of fetus has been shown to have a definite and significant influence in determining the length of time between breeding and parturition.

In most dairy (17, 101, 130) and some beef breeds (23, 92), gestation length is slightly longer (1 to 2 days) for male than for female calves. Because this difference is small, some investigators have been unable to show a significant effect of sex of the fetus on the mean duration of pregnancy. In the most recent studies, however, in which records from a large number of animals were analyzed, significant differences have been demonstrated (17, 130). McKeown and MacMahon (101) reinvestigated data published by Spencer (144) and Knott (94) and found a marked increase in ratio in favor of male calves as the length of pregnancy increased. Furthermore, using these same analytical

methods, they confirmed other observations that, in contrast to the cow, the gestation period of humans was shorter for males than for females. This difference they considered was at least partially attributable to the greater rate of prenatal growth of male children; the earlier delivery of the male fetus being due to its greater fetal weight. The relatively small weight differences between the sex of fetus, however, may be unimportant when one considers that heavier calves are usually associated with longer pregnancies.

An alternative explanation based on a fetal endocrine sex difference should also be considered.

As early as 1909, Von Oettingen (157) presented data that sex of fetus influenced gestation length in the mare, a finding supported by other studies (107, 116, 146, 153) in which small but highly significant differences were found. The males were carried 1.6 to 1.7 days longer than the females. This difference due to sex, however, has not always been observed (20, 32, 75).

c. Order of Pregnancy and Age of Dam. The length of pregnancy in the rabbit is only slightly affected, if at all, by age of dam or order of pregnancy (115, 131). In other species, notably the goat (9), the pig (85, 95), and the sheep (15), sequence of pregnancy also appears to have little or no influence on gestation length. Although the number of previous pregnancies has been reported to influence gestation length in the mare (157), Britton and Howell (20) could find no important differences in duration of this period between primiparous and pluriparous animals.

Agreement on the effect of the age of the cow on gestation length has not been consistent. Brakel *et al.* (17) cited, in their review, the work of five authors who found no influence of age of dam and an equal number who reported differences ranging from 3 to 7 days between the mean gestation lengths of 2-year-old and mature cows. From their own records, they found that the mean gestation lengths of cows five years old and over significantly exceeded that of 2-year-old cows by 1.5 days.

The results obtained by Rollins *et al.* (130) suggest that the number of pregnancies associated with age was an important factor in the gestation period of cattle. First calves were carried 1.3 days less than second calves; second calves were carried 1.3 days less than third calves. From the third calving sequence on, no significant difference was found. They state that age, per se, was not the contributing factor, but, in reality, the apparent effect of age reflects a variation in stress conditions associated with management practices and whether or not a cow was lactating when pregnant. Furthermore, the existence of a positive cor-

relation between milk production and gestation length of the current pregnancy tends to support the idea that an endocrine influence may be operating.

In the goat, the age of the dam has some influence on gestation length. It is least for first year conceptions and rises gradually to a maximum of 151.3 days at 6 years (8).

Coburn (29) reported that, in pigs, older sows have longer gestation periods (112–115) than younger ones (100–106 days at time of first litter), but, in subsequent analyses, this influence of age could not be shown (95).

A slight effect of age of mare upon the length of time she carries her fetus has been indicated, but whether this is a significant contribution is open to question (75, 107).

In the ewe, an average increase of 0.27 days in the mean length for each advancing year of age was found by Terrill and Hazel (150). This effect of age was not due to weight differences since weight at breeding time had no influence.

Weight as a factor in determining the time the fetus spends *in utero* has not been studied in most species; because of the influence of other variables, the importance of weight of the dam is difficult to ascertain. Only one study, conducted on cattle, reported that the gestation length of heavier cows exceeded that of light ones (77).

With few exceptions, observations on the domestic species indicate that sex of fetus, size of litter, and age or parity of dam may all influence gestation length. At what point in the period of pregnancy they exert their effect is not clear. An action of the fetus on the initiation of parturition by an alteration of the uterine environment either through physical, metabolic, or hormonal means is possible. A difference in the interval between mating and fertilization has been suggested. Effects, such as a delayed implantation of the blastocyst, which are associated with the maternal hormone environment must also be considered. In this respect, Rollins *et al.* (130) found a positive correlation between milk production of the cow and gestation length and postulated an endocrine influence. Other contributing factors, such as age or parity of the dam, must be related to differences in the internal environment as a result of anatomical as well as endocrine changes.

III. PROLONGED GESTATION

From the foregoing discussion of factors influencing the length of gestation, it is apparent that, before parturition can be considered unduly prolonged, a normal range in the duration of pregnancy for each species must be defined.

A reference to Table I indicates that, in the domestic species, with the exception of the cow and possibly the mare, the maximum does not greatly exceed the mean gestation period. One report on the gestation length in the rabbit mentioned instances of prolonged pregnancy up to 55 days (115).

In humans, Martius (105) reported that about 10% of the women in his clinic had periods of pregnancy of more than 296 days. Although he does not so state, it is presumed that this period is the interval from the cessation of menstruation to labor.

In the extensive bibliography compiled by Kenneth (90), eleven references are cited of human pregnancies lasting from 308 to 351 days. The failure to establish a definite time of fertilization makes it difficult to ascertain the correctness of these estimates, particularly those indicating extreme overterm. Furthermore, the fact that in many cases of prolonged gestation in the human, infants are not excessively large, leads to additional skepticism. Nevertheless, an abnormally extended period of pregnancy in the human probably does occur, but must be considered infrequent.

The overlong duration of pregnancy occasionally found in the mare may be associated with pathological factors, principally intrauterine infections (20, 160). Dead or diseased foals from mares with periods longer than 340 days are frequent.

Among the most interesting and well-documented cases of prolonged gestation are those which occur in cattle.

Andres (6) gave partial descriptions of 53 cases of prolonged gestation in cows. Most of the calves were males; all were gigantic and several had fetal diarrhea and contained hair balls in the amniotic fluid. Similar cases of cows with prolonged pregnancies have been reported by others (57, 60, 80, 89, 100, 118, 143, 148, 160).

Gregory *et al.* (57) traced the ancestry of 30 overterm Holstein calves to a single bull and concluded that this defect was conditioned by a single autosomal recessive gene. The genotype of the fetus clearly was a decisive factor in determining prolongation. Thus, the same cow was capable of producing normal calves as well as prolonged.

The clinical and pathological features of the condition in the same herd have been described in some detail (80). Most of the 30 calves were female, but the fact that 9 male fetuses were involved suggests that sex is not a significant factor in causing the particular condition. In those cases where the cow was allowed to continue pregnancy until labor occurred, the time overdue ranged from 20 to 88 days. Post-term growth was apparent; the weights of the calves were large, varying

from 105 to 168 pounds; the hair was thick, long and abundant; the incisors were large; and hair was present in the digestive tract as well as the amniotic fluid.

Labor in most of the cows was extremely difficult due to the very large size of the calf. Forced extraction or embryotomy was usually necessary to complete delivery. In those cases where caesarean section was done, some calves were delivered alive, but all died within eight hours of birth. These deaths are not associated with the length of intra-uterine existence. Live calves obtained from a cow 40 days overdue, a cow 20 days overdue, and one 12 days overdue, all died within a few hours of birth. Post-mortem examination revealed no gross abnormalities to explain the cause of death. In one case in which a prolonged calf was maintained by chronic intravenous infusion of 5% glucose and "nipple-fed" colostrum, the animal lived for almost 4 days. Death resulted following treatment withdrawal and was associated with a steady fall in the level of blood glucose (79).

A general characteristic of cows carrying prolonged calves was the failure to display the usual signs of parturition. Udders were comparable to those of animals in their 32nd to 34th week of gestation, enlargement frequently being delayed until the day of parturition. Relaxation of the pelvic ligaments and relaxation and edema of the vulva were lacking or occurred only to a mild degree. Cervical gland mucus was absent, leaving the birth canal "dry" (79).

Cows surviving the operation began secretion of significant amounts of milk within a week; thereafter milk flow gradually increased and some became excellent producers. Colostrum was not recognized.

As in the Holstein breed, the excessively long gestation period observed in Guernsey cows is also associated with a genetic defect. The anomaly likewise may depend upon a single autosomal recessive gene (148).

The pathological characteristics of the fetus in Guernseys differ markedly from those in Holsteins. In spite of the excessive duration of pregnancy, the calves are small, ranging in weight from 40 to 70 pounds. Kennedy *et al.* (89) described 10 fetuses obtained from a single herd. Some showed a complete absence of hair, others had areas of some hair development varying from a small patch to a complete coverage. The crania were characteristically domed, the legs disproportionately short, but the hooves were large. Linear skeletal growth of the long bones was retarded, and estimates based on size, weight, and hair coat indicated a fetal maturity of about 7 months. Other abnormalities included atresia of the jejunum and extreme facial malformations, which

in one case qualified the calf as a pseudocyclopean monster. An aplasia of the adenohypophysis was a characteristic finding in all the abnormal fetuses examined, but the neurohypophysis was present except in two monsters. Thyroids were small, and serum protein-bound iodine reduced. The adrenal cortical zones were not differentiated. The ovaries appeared essentially normal, but no microscopically detectable Leydig cells could be found in the testes. The authors suggested that the arrested fetal development was probably associated with the lack of a "tropic" hormone influence as a consequence of anterior pituitary agenesis.

As in the Holstein-Friesian cows, the Guernseys, long after expected delivery, were similar in appearance to normal pregnant animals in the seventh month of gestation. Parturition, however, did not occur until shortly after the fetal death, which presumably furnished the conditions necessary for expulsion of the uterine contents. Prior to the beginning of labor, the characteristic changes in the mammary glands did not occur. Following the delivery of the fetuses, the udder developed rapidly, and milk secretion was essentially normal in about 10 days. No colostrum was noted.

These genetic anomalies are most interesting from the standpoint of a study of factors initiating parturition. The fetal developmental arrest which has been observed in the Guernsey breed seems to point to the influence of fetal mass as a contributing factor initiating the normal birth mechanism. That this, alone, cannot be the specific stimulus, at least in the cow, is clearly indicated by the association of excessively large fetuses with prolonged gestation in the Holstein breed. There is no known explanation for the occurrence of the condition in these animals, although some have speculated on an alteration of the endocrine relationships (57).

IV. PARTURITION

By this time it is apparent to the reader, not only from the foregoing discussion, but also from accounts in Chapters 6 and 14, that the factors involved in the maintenance of pregnancy are multiple.

Parturition, therefore, must presume the gradual elimination of these factors and probably is the result of the culmination of structural, hormonal, nervous, nutritional, and circulatory influences, no one of which can be considered as *the* specific stimulus for the beginning of labor.

The statement that the mechanism of parturition is a mystery is not entirely valid, for, indeed, much knowledge has been accumulated. A

difficulty arises, however, when one attempts to fit all the known factors involved into a clear and concise picture

Before a consideration of these elements can be undertaken, it is necessary to describe the physical and morphological changes occurring at term

A Sequence of Events in Normal Labor

Most textbooks customarily subdivide parturition into three stages or phases

1 First Stage

This stage is characterized by the dilation of the cervix and active contractions of the longitudinal and circular muscle fibers of the uterine wall Reynolds (122) has described in considerable detail the development of factors activating the uterus at term Under the influence of estrogen, growth of the uterus is retarded and the myometrium becomes irritable and contractile As pregnancy advances, there is a progressive diminution of fetal fluids, which are replaced by the solid body of the fetus Coincident with this decrease in fluid, an increased uterine pressure develops The uterus, now able to contract against a solid body, creates an expulsive force As a result of limitation of uterine growth and in the presence of a rapidly developing fetus, an increased tension, which is approximately three times greater in the fundus than in the lower uterine segment and cervix, develops As pregnancy progresses, the upper uterine segment becomes more resistant to change of shape due to the hypertrophied elastic and muscle tissue In contrast, this increased proliferation of connective tissue does not occur in the lower segment These differentials in resistance and tension between the fundus and the lower uterine segment, including the cervix, create a pressure gradient that favors expulsion of the uterine contents

Now the strong rhythmic contractions of the longitudinal muscle fibers of the fundus, occurring about every 15 minutes and lasting 15 to 30 seconds, cause a retraction of the tissues of the lower segment The cervix, as a consequence, becomes effaced and dilated Aiding in the process of dilation are mechanical, wedgelike actions of the fluid-filled membranes, as well as the head or other parts of the fetal body

In uniparous animals, such as the cow and mare, the contractions start at the apex of the cornua while the caudal part does not contract In multiparous animals, the contractions of the uterus occur just cephalad to the most caudal fetus while the rest of the uterus remains quiescent (124). A separation of the fetuses *in utero* has been observed

in the rabbit; the uterine canal is very thin-walled where it is extended by the fetus, but, between each fetus, the musculature is condensed and thick, making the lumen negligible (53).

In the cow and ewe, the first stage may last from $1/2$ to 24 hours; in the mare, about 1 to 4 hours; and in the sow, between 2 to 12 hours.

2. Second Stage

This stage is called the expulsive phase and is characterized by the complete dilation of the *os uteri*, the entrance of the fetus into the birth canal or pelvis, contraction of the abdominal diaphragmatic muscles together with a closing of the glottis, and the final delivery of the fetus through the vulva.

During this phase, the frequency and duration of the uterine contractions are increased. In the cow, they appear about 7 times in a 15-minute interval, and last about a minute and a half (124). According to Benesch, cited by Roberts (124), the intrauterine pressure in the cow during the first stage of labor was 66 mm.Hg between and 99 mm.Hg during uterine contractions. In the second stage, a pressure of 170 mm.Hg was recorded during the time of abdominal contraction. In the human, during delivery of the head, the expulsive force has been estimated to be as much as 15 kg. (165). The contractions of the muscle of the abdomen and diaphragm occur synchronously with those of the uterus and may be initiated by pressure stimuli in the birth canal. In pregnant rabbits, mechanical pressure applied to the narrow part of the canal between the symphysis pubis and the terminal portion of the spinal cord caused strong parturitional efforts of the skeletal muscle (53). In humans, early in labor, these contractions are to some extent voluntary, while later they become involuntary (165).

In cows, the duration of this second phase is from $1/2$ to 4 hours. In mares, it is quite short, lasting from 10 to 30 minutes. The period in ewes and goats is completed in $1/2$ to 2 hours; in sows, it lasts from 1 to 4 hours. Primipara usually take longer than pluripara.

Perry (114) has given an interesting account of the sequence of events in parturition in the pig. Early in the birth process, the ends of the chorionic sacs rupture and form a slippery lining, lubricated with mucus and closely adherent to the endometrium. Movements of the piglet against the partially degenerated amnion probably cause this rupture and help bring about the detachment between the amniotic and allantoic layers. As a result, the pig is allowed free passage along the course of the uterine cavity (141). Their placental connections, however, are retained through the very extensible umbilical cord. Separ-

tion may not occur until after birth, when the pig, in an effort to suckle, attempts to pull away. Fetuses are not necessarily born in order of their position in the uterus, or with regard to their location in the right or left horn.

3. *Third Stage*

This phase encompasses the expulsion of the placenta, and is a complex process involving both mechanical and hormonal factors. The exact mechanism has not been completely elucidated.

As a result of a diminished efficiency of the circulation of maternal blood within the placenta, it begins to degenerate with the advance of pregnancy (122). In the human, beginning thrombosis of many of the large venous sinuses of the placenta and an obstruction of the lumens of the vessels by giant cells can be observed by the eighth lunar month of pregnancy (35, 98).

Complete degeneration of the fetal placenta is accomplished with the birth of the fetus. The vessels collapse with the severance of the umbilical cord, and the villi become shrunken. The continued contraction of the uterus reduces the amount of circulating blood in the endometrium and forces the placenta into the birth canal. As a result of the reduced blood volume, the maternal crypts relax. This effect, together with the collapse of the anchoring villi, facilitates the loosening of the chorionic attachments, resulting in the separation of the maternal and fetal placenta. The weight of the amnion and a portion of the allantois may aid in the final removal of the placenta.

In multiparous animals, the fetal membranes are not necessarily expelled coincident with the birth of each fetus. For instance in the sow, since the allantois-chorions may be fused, it appears probable that all or most of the young are delivered before the membrane is shed (114).

The time for complete removal of the fetal membranes in the cow and the ewe is normally $1/2$ to 8 hours. In the mare, this period lasts only a short time ($1/2$ to 3 hours), possibly due to the nature of the loose attachment of the diffuse epitheliochorial placenta.

Following parturition, involution of the uterus takes place within a varying length of time, dependent upon the species. In the mare, this recovery process proceeds at a fairly rapid rate; by 13 to 25 days after birth the endometrium is restored to normal (7). As conception occurs frequently 8 to 12 days postpartum, the restorative process must be essentially complete at this time (124).

According to some authors, involution of the uterus of the cow occurs within 26 days following parturition (24, 119), while others consider

that an average of 47 days is more nearly correct (22, 124). Involution of the uterine mucosa of the ewe has been reported to be complete at about 30 days after delivery of young (154).

B. Biochemical Changes in the Placenta at Term

Before severance of the cord, the fetus is entirely dependent on the maternal organism for its supply of nutrients and for the elimination of those waste products it cannot store. The placenta is admirably suited to play a major role in this exchange. Furthermore, since a gradual degeneration of this tissue appears to take place during the later stages of gestation in certain species, e.g., man, it is pertinent to consider briefly the associated biochemical changes. Possibly the withdrawal of placental influences which stabilize pregnancy or initiate parturition may be related to these degenerative changes.

In two recent symposia, certain aspects of placental morphology and function have been well reviewed (31, 76). Although a treatment of this subject is beyond the scope of this discussion, it is surprising how few observations have been made on the changes in the biochemistry of the placenta specifically associated with the stages of gestation. In humans, from the seventh to the eighth month, the volume of the placenta increases more rapidly than the dry weight, in contrast to the inverse relationship during the first seven months (41, 158). Similarly at this late stage, the content of the amino acids proline, oxyproline, tryptophan, and particularly arginine declines (41, 158); coincidentally, permeability decreases (49). Throughout pregnancy, a gradual reduction of oxygen consumption and glycogen content takes place (155).

Choline (27, 106), acetylcholine (26), and cholinesterase (152, 163) have been found in human placental tissue. Although acetylcholine may stimulate contractility of muscle tissue and the threshold of its excitation can be affected by sex hormones (152), its exact relationship to the phases of active uterine contractility associated with parturition has not been determined.

The presence of hormones in placental tissues is well documented. As this subject has been reviewed in the previous chapter, it will not be discussed here. Placental hormonal content in the domestic species during different periods of gestation, however, has not been studied systematically, with the exception of gonadotropin. Investigations in this regard would be fruitful.

V. THE INITIATION OF PARTURITION

Diverse, but related, factors influence the initiation of parturition. Indeed, no one specific stimulus appears to be effective; rather, nervous,

endocrine, and physical elements all converge synchronously to bring about the final act of labor. To simplify presentation, each of these factors will be treated separately, but one should keep in mind that each represents only a part of a well integrated mechanism.

A Neural Factors

Although the neural factors participating in parturitional events are discussed in Chapter 6, a brief summary in this section is pertinent.

The early observations that normal delivery of litters following spinal cord transection in dogs by Goltz and Freusberg (55), since confirmed by others (11, 120), clearly demonstrates that parturition can occur independently of the central nervous connections. Similarly, normal labor has been described in humans with spinal injuries involving the destruction of the cord at levels from the seventh cervical vertebra to the lumbar region (42, 132, 149), and even the isolated, completely denervated human uterus is capable of expelling a fetus (10).

These facts would appear to rule out an important role of extrinsic neural agents on the mechanism of parturition. Nonetheless, the existence of a spinal center at the level of the tenth thoracic to the second lumbar segment to regulate and coordinate contraction and retraction of the uterus during labor has been postulated (132).

The presence of an intrinsic innervation of the uterus can be demonstrated anatomically (21, 48, 87), but no evidence has been provided to substantiate its functional relationship to the activity of uterine muscle during labor (122).

Much better evidence exists for a reflex release of oxytocin from the neurohypophysis by way of an afferent nervous pathway in response to stimulation of the vagina or cervix. This neuroendocrine relationship may be of physiological importance in the initiation of uterine motility, but the results of many experiments involving posterior lobe removal, spinal cord transection, or lesions of the supraoptic nucleus indicate that an intact system is not essential for the birth mechanism (Chapter 6) (62).

The state of our present knowledge with respect to specific neural agents affecting parturition can be aptly summarized by quoting from a statement by Reynolds (122), "It seems unlikely that the numerous and complex nervous connections with which nature has equipped the uterus are merely structural ornaments. Yet with few exceptions, chiefly those concerned with the sensory aspects of uterine function, every reproductive and sex function about which we know can be subserved without the agency of these nervous structures."

B. Endocrine Factors

Among the first experimental investigations on the existence of blood-borne agents active in the role of parturition are those of Sauerbruch and Heyde (133). From observations on conjoined human female twins, they noted that, following the pregnancy of one, breast development occurred in both; they concluded that active agents, produced as a result of pregnancy, were transferred from one organism to the other by the blood stream. To test their hypothesis, they parabiotically united pregnant female rats during different stages of gestation. When females in early pregnancy were united with animals near term, in some cases no influence was noted on the normal duration of pregnancy of either; in other instances, in the early pregnant partner, symptoms of intoxication occurred and were associated with abortion. To account for these effects, they postulated that toxic substances, against which the animal near term was protected, were produced at the end of pregnancy. That these factors are associated with pregnancy seems doubtful, since it is now well known that "parabiotic intoxication" may occur as a result of partner incompatibility (46). When Kross (96) repeated these experiments on fully mature pregnant rats which were selected for union so that the gravidity of one was much more advanced than the other, labor occurred at the normal time in all except one of the surviving pairs. Clinical evidence obtained following the injection of human fetal serum into preparturient women suggested that parturition was hastened. Based on these observations, von der Heide (68) reasoned that parturition was caused by the production of maternal antibodies or "birth materials" against the increased concentration of antigens from the fetus.

Knowledge accumulated concerning the role of hormones in the maintenance of pregnancy provides other possibilities of interpreting these early experiments. Parturition probably entails a withdrawal or alteration of these stabilizing influences, as well as an increased production of other chemical substances capable of stimulating uterine muscular activity or causing a relaxation of the lower uterine segments and the pelvic region.

An explanation of parturition based upon the withdrawal of hormones has had many adherents. Much can be said in support of this concept. The termination of pregnancy following the removal of the ovary or corpus luteum and changes in hormone levels of progesterone or its metabolic product, pregnandiol, during pregnancy have been discussed in detail in Chapter 14.

The progesterone content of human placenta is reported to be higher before labor than after (63), and pregnandiol levels are unusually low

shortly before abortion (16). A prolongation of pregnancy by the chronic administration of progesterone (67, 111), or by maintenance of luteal function and progesterone secretion by injecting gonadotropins (74, 142) is well established. These facts indicate that parturition must depend in part, at least, upon the withdrawal of progestins.

On the other hand, when blood levels of progesterone were measured during pregnancy in the rabbit (167), in the mouse (50), and in the ewe (109) by the Hooker-Forbes test (73), no drop in its concentration could be detected until after parturition was completed. Attempts to detect progesterone in pregnant sheep blood by chromatographic techniques with a sensitivity of 0.1 $\mu\text{g./ml.}$ have been unsuccessful (40).

One explanation for these paradoxical findings is the possible existence of another progestin with a higher biological activity than progesterone. Recently Short (134) has identified a substance in the peripheral blood of sheep identical with 20-hydroxypregnenone. This material possesses a high degree of biological activity by the Hooker-Forbes test, and may be the substance responsible for the persistent progestational activity of pregnant ewe serum, following parturition. If this is true, then the assumption of the withdrawal of progestational compounds as the single specific stimulus for the initiation of parturition may not be the case, since disappearance of activity should precede labor.

Strips of uterine tissue nearest the placenta are more responsive to progesterone than those from a distance (33); thus, the possibility of local changes in uterine sensitivity is suggested. Measurements of peripheral blood levels, therefore, cannot necessarily be interpreted unless the associated physiological changes taking place within the uterus are also considered.

It is well known that estrogens exert a stimulating action on the myometrium (52, 126, 127). Similarly, this rising level of estrogen in the blood with the advance of pregnancy probably accounts for the increase in spontaneous contractile activity of the uterus (136). These facts have prompted the suggestion that the concentration of this hormone at term might be a specific cause for the initiation of labor. The fact that the administration of estrogenic substances will terminate pregnancy in rats, guinea pigs, cows, and sheep provides additional support for such a concept (28, 88, 137). In pregnant humans, however, large quantities of estrogen do not have this effect (99, 127).

It is doubtful that estrogen is the only entity involved in the initiation of parturition; rather, it may play a major role by increasing the irritability of uterine muscle to other stimuli (121). To this extent, n

relationship of estrogen to progesterone is clearly indicated. It is now generally recognized that estrogen must act upon the uterus before progesterone can be effective (97); further, normal pregnancy development requires a certain ratio between these two compounds (135). Progesterone inhibition of the spontaneous contractile activity of the uterus following estrogen treatment provides additional evidence of the importance of the ratio of these hormones in determining the nature of the response (5, 43, 128). It appears, therefore, that some optimal relationship between these two substances must exist to create the conditions necessary for parturitional events. When the level of only one is considered quantitatively, its relationship to labor is difficult to interpret.

The sensitivity of the uterine muscle to oxytocin is usually increased under the influence of estrogen and in many species depressed by progesterone (93, 122, 125); this provides additional evidence of the importance of the relationship between these two compounds in parturition.

In a recent symposium, Fitzpatrick (47) presented data supporting the concept that parturition follows a coordinated pattern for different parts of the uterus. By recording intracervical coincident with intra-uterine pressures in pregnant cows, he showed that the magnitude of the response of the *uteri corpus* to oxytocin increased gradually throughout most of the second half of pregnancy and was greatly accelerated just before parturition. In the cervix, however, the reactivity remained constant and even diminished. At mid-pregnancy, on the other hand, the cervical response was dominant, but decreased in late pregnancy. From these results, he concludes that uterine irritability during mid-pregnancy favors retention of the fetus while that of late pregnancy favors expulsion.

Much can be said to support the important role of oxytocin in parturition. Thus, in the postparturient rabbit, electrical stimulation of the hypophyseal stalk results in increased uterine activity (64), and stretching of the cervix causes a release of oxytocic substance from the pituitary gland (45). After parturition, oxytocin is depleted from the neurohypophysis in rats and dogs (2, 37), and presumably, as indicated by the expression of milk during uterine contractions (58), is liberated into the circulation during labor in women.

On the other hand, results of hypophysectomy or posterior lobe removal have been variable. Ablation, after conception, of the posterior lobe in rats and rabbits (51, 138), or complete hypophysectomy in monkeys (140) resulted in no disturbance in gestation length or parturi-

tion. Other findings indicate prolonged labor following excision of this gland or electrolytic destruction of the pituitary stalk (36, 113, 139).

These surgical techniques do not eliminate the possibility of an important role in parturition of substances produced by the hypothalamus. The importance of a reflex release of these compounds, however, by neural mechanisms may be questioned in view of the failure of the elimination of the sensory components of uterine nervous innervation to affect the course of events leading to fetal expulsion.

Information on the effectiveness of oxytocin in inducing parturition in domestic animals is meager, but one study shows that oxytocin is capable of inducing labor in the sow (108). Assays of fetal pig and sheep pituitaries indicate that the maximum concentration of oxytocin is reached during the latter stages of gestation just before parturition (13). The extensive studies on the effects of fetal decapitation *in utero* have not included a description of parturitional derangement (84). To determine an effect of the fetal pituitary on the initiation of parturition, this technique might be useful when applied to animals bearing single young or animals in which all other living young have been surgically removed to exclude an influence by the hypophyses of the intact fetuses. Congenital absence of the pituitary gland in human fetuses is not necessarily associated with a derangement in parturition (19), but, as discussed earlier, in cows, adeno-hypophyseal aplasia of the calf is characteristically found in cases of abnormally long gestation periods. A neurohypophysis was, nevertheless, present (89). Furthermore, placentas, after the early ablation of the embryos, are delivered at the expected time of parturition (156).

Our present knowledge on the role of fetal endocrines in parturition is too meager to hazard an interpretative conclusion, and evidence supporting the involvement of the fetal pituitary is lacking.

Another important site of formation of oxytocic material is the placenta, from which extracts highly effective in stimulating uterine contractility have been obtained (34).

The presence of a substance in the blood of pregnant women capable of inactivating oxytocin has been repeatedly verified (44, 65, 164). Recently, by careful investigations, Dickler and Tyler (38) were able to demonstrate that nonpregnant human plasma is incapable of inactivating oxytocin, whereas mid-pregnant plasma contains some "oxytocinase" activity which is gradually lost during the last few weeks before term. A persistence of "oxytocinase" potency was associated with prolonged labor. In a recent report, these results could not be confirmed (68). It seems somewhat premature to assign a significant role

implying the withdrawal of "oxytocinase" as a factor precipitating parturition; its presence in pregnancy plasma only suggests a functional relationship to the maintenance of gestation.

In addition to estrogen, progesterone, and oxytocin, a fourth hormone, relaxin, must surely be involved in normal parturition. The well-established effect of this hormone on the phenomenon of pubic relaxation at term in estrogen-primed rodents illustrates this association (70). Additional physiological effects of relaxin have been reviewed by Hisaw and Zarrow (72). These include a deciduomata-inhibiting effect on the rat uterus, a synergism with estrogen and progesterone on the lobulo-alveolar development in rat mammary tissue, antidiuretic and pregnancy anemia effects in rabbits, and an inhibition of the spontaneous motility of the guinea pig uterus. This latter effect may explain the value of relaxin in the clinical treatment of the threatened premature labor in the human (1).

Recent investigations in the rat have reported a synergism of relaxin with estrogen on uterine hypertrophy (78). In the ovariectomized mouse treated with progesterone, relaxin causes an increase in the responsiveness of the uterus to oxytocin in the latter stages of pregnancy and brings about the punctual and normal delivery of young (59, 147).

Although remarkably high concentrations of relaxin can be obtained from the pregnant sow's ovary (71), few studies have been reported on its physiological role in domestic animals. Most of the work up to the present time has been confined to the rabbit, guinea pig, and human. In these species, there is a significant increase in its concentration during pregnancy (102, 166, 168).

From the interesting investigations of Zarrow (170), the ovary, uterus, and, particularly, the placenta of the rabbit appear to be capable of producing relaxin.

Relaxation of the cervical muscles of the cow following varying dosages of relaxin is possible if these animals are first pretreated with estrogen (56). Similarly, dilation of the uterine cervix of the castrated gilt pretreated with diethylstilbestrol can be demonstrated following treatment with 15,000 to 36,000 G.P.U. of relaxin. Neither diethylstilbestrol nor progesterone, either singly or in combination, is capable of producing this effect. No changes in the symphysis pubis of the gilt occurs following relaxin treatment, but histochemical and gross histological studies of the uterine cervix show a depolymerization and an edema. Furthermore, the vulvar changes following this treatment are comparable to those occurring normally in the sow at term (169).

These results following relaxin treatment in the estrogen-primed cow

and sow are reminiscent of the characteristic changes signifying impending parturition in these species. In both, a relaxation and edema of the vulva are apparent and, in the cow, relaxation of the pelvic ligaments usually precedes birth by only a few hours.

Detailed studies on sheep slaughtered at different stages of pregnancy revealed no relaxation in the pubic symphysis at any stage, but, between the second and third months, the sacroiliac joints and ligaments had begun to loosen. A relaxation of the ligaments can be produced in ovariectomized ewes by estrogen alone, but whether relaxin synergizes in this response has not been investigated (12).

C. Physical Factors

The effect of stretching resulting from an increase in fetal mass on uterine irritability and tension has been discussed. Furthermore, we have seen that fetal size or litter number contributes to changes in the length of gestation. For example, the fetal developmental arrest as a result of the congenital absence of the anterior pituitary is associated with an excessively long gestation period in the Guernsey breed; twins are usually born earlier than singles; and when fetal growth is retarded by inadequate nutrition, parturition is delayed. These observations certainly suggest a contribution of physical factors to the events leading to birth. Indeed, the continually developing conceptus cannot be accommodated indefinitely by the uterus. Eventually the nutritional supply to the fetus by way of the maternal vascular bed must become inadequate; when this time is reached, the fetal-maternal relationship is interrupted and pregnancy terminated.

In summary, it is apparent that the initiation of parturition cannot depend upon a single specific stimulus. Rather it is an extremely complex event, probably involving many factors. Nervous stimuli, known and unknown chemical substances, such as hormones, as well as physical influences all may integrate to bring about the proper physiological changes required for normal birth.

REFERENCES

1. Abramson, D., and Reid, D. E., *J. Clin. Endocrinol. and Metabolism* **15**, 206 (1955).
2. Acher, R., and Fromageot, C., in "The Neurohypophysis" (H. Heller, ed.), p. 48. Academic Press, New York, 1957.
3. Alexander, C., *Nature* **178**, 1058 (1956).
4. Alexander, M. H., *J. Dairy Sci.* **33**, 337 (1950), abstract.
5. Allen, W. M., and Reynolds, S. R. M., *Science* **82**, 155 (1935).
6. Andres, J., *Deut. tierärztl. Wochenschr.* **39**, 567 (1931).

7. Andrews, F. N., and McKenzie, F. F., *Missouri Univ. Agr. Exptl. Stat. Bull.* No. 329 (1941).
8. Asdell, S. A., "Mammalian Reproduction." Comstock, Ithaca, New York, 1946.
9. Asdell, S. A., *J. Agr. Sci.* 19, 382 (1929).
10. Balard, P., *Compt. rend. soc. biol.* 82, 1113 (1919).
11. Bard, P., *Psychosomat. Med.* 4, 171 (1942).
12. Basset, E. G., and Phillips, D. S. M., *Nature* 174, 1020 (1954).
13. Bell, C. H., and Robson, J. M., *Quart. J. Exptl. Physiol.* 27, 205 (1938).
14. Bonadonna, T., and Valerani, L., *Zootec. e vet.* 1, 129, 274 (1946). *Animal Breed Abstr.* 15, 174 (1947).
15. Bonsma, F. N., *Univ. Publ. Pretoria Ser. 1 Agr.* 48 (1939).
16. Bourgarel, R., and Ferrante, L., *Bull. fédération soc. gynécol. et. obstét. langue franç* 3, 239 (1951).
17. Brakel, W. J., Rife, D. C., and Salisbury, S. M., *J. Dairy Sci.* 35, 179 (1952).
18. Braude, R., and Walker, R. M., *J. Agr. Sci.* 39, 156 (1949).
19. Brewer, D. B., *J. Pathol. Bacteriol.* 73, 59 (1957).
20. Britton, J. W., and Howell, C. E., *J. Am. Vet. Med. Assoc.* 102, 427 (1943).
21. Brown, W. H., and Hirsch, E. F., *Am. J. Pathol.* 17, 731 (1941).
22. Buch, N. C., Tyler, W. J., and Casida, L. E., *J. Dairy Sci.* 38, 73 (1955).
23. Burris, M. J., and Blunn, C. T., *J. Animal Sci.* 11, 34 (1952).
24. Casida, L. E., and Venzke, W. G., *Proc. Am. Soc. Animal Production 29th Meeting*, 221 (1936).
25. Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.* 55, 167 (1934).
26. Chang, H. C., Lee, L. Y., Meng, C. W., and Wang, Y. K., *Proc. Soc. Exptl. Biol. Med.* 49, 380 (1942).
27. Chang, H. C., *Proc. Soc. Exptl. Biol. Med.* 32, 1001 (1935).
28. Clegg, M. T., unpublished observations, 1958.
29. Coburn, F. D., "Swine Husbandry." Orange Judd, Co., New York, 1919.
30. Cochrane, R. L., and Meyer, R. K., *Proc. Soc. Exptl. Biol. Med.* 96, 155 (1957).
31. Cold Spring Harbor Symposia Quant. Biol. 19 (1954).
32. Cortez, E., *Rev. militar Remonta Vet.* 10, 149 (1952).
33. Csapo, A., *Am. J. Anat.* 98, 273 (1956).
34. De Boer, S., Dreyer, N. B., and Clark, A. J., *Arch. intern. pharmacodynamic* 30, 141 (1925-1926).
35. De Snoo, K., *Monatsschr. Geburtshilfe u. Gynäkol.* 57, 1 (1922).
36. Dey, F. L., Fisher, C., and Ranson, S. W., *Am. J. Obstet. Gynecol.* 42, 459 (1941).
37. Dicker, S. E., and Tyler, C. M., *J. Physiol. (London)* 121, 206 (1953).
38. Dicker, S. E., and Tyler, C. M., *J. Obstet. Gynaecol. Brit. Empire* 63, 690 (1956).
39. Dry, F. W., *New Zealand J. Agr.* 47, 386 (1933).
40. Edgar, D. G., *J. Endocrinol.* 10, 54 (1953).
41. Ehrenberg, R., and Liebenow, W., *Arch. ges. Physiol. Pflüger's* 201, 387 (1923).
42. Elkin, D. G., *J. Am. Med. Assoc.* 78, 27 (1922).
43. Evans, E. I., and Miller, F. W., *Am. J. Physiol.* 116, 44 (1936).
44. Fekete, K. von, *Endokrinologie* 7, 364 (1930).
45. Ferguson, J. K. W., *Surg. Gynecol. Obstet.* 73, 359 (1941).
46. Finerty, J. C., *Physiol. Revs.* 32, 277 (1952).
47. Fitzpatrick, R. J., in "The Neurohypophysis" (H. Heller, ed.), p. 203. Academic Press, New York, 1957.

- 48 Fleming A M, *J Obstet Gynaecol Brit Empire* 35, 247 (1928)
- 49 Flexner, L B, Cowie, D B, Hellman, L M, Wilde, W S, and Vosburgh, G J, *Am J Obstet Gynaecol* 55, 469 (1958)
- 50 Forbes, T R, and Hooker, C W, *Endocrinology* 61, 281 (1957)
- 51 Fortgang A, and Simpson M E, *Proc Soc Exptl Biol Med* 84, 663 (1953)
- 52 Frank, R T, Bonham, C, and Gustavson, R C, *Am J Physiol* 74, 395 (1925)
- 53 Franklin K J, and Winstone, N E, *Am J Physiol* 125, 43 (1954)
- 54 Gerlaugh, P, Kunkle, L E, and Rife, D G, *Ohio Agr Exptl Stn Research Bull* 703 (1951)
- 55 Goltz, F, and Freusberg A, *Arch ges Physiol Pfluger's* 9, 552 (1874)
- 56 Graham, E F, and Dracy, A E, *J Dairy Sci* 36, 772 (1953)
- 57 Gregory, P W, Mead, S W, and Regan W M, *Portugolhoc Acta Biol Sér A, R B Goldschmidt Vol* p 861 (1951)
- 58 Gunther, M, *Brit Med J* 1, 567 (1948)
- 59 Hall, K, *J Endocrinol* 15, 108 (1957)
- 60 Hallgren, W, *Nord Veterinarmed* 3, 1043 (1951), *Vet Bull Commonwealth Bur Animal Health* 22, 550 (1952), abstract
- 61 Hammond, J, and Marshall, F H A, *Wiss Ber Weltgeflugelkong* 1, 153 (1936)
- 62 Harris, G W, *Arch Gynokol* 183 35 (1953)
- 63 Haskins, A L, *Am J Obstet Gynecol* 67, 330 (1954)
- 64 Haterius, H O, and Ferguson J K W, *Am J Physiol* 124, 314 (1938)
- 65 Hawker, R W, *J Endocrinol* 13 vP (1955)
- 66 Hawker, R W, and Robertson, P A, *Endocrinology* 60, 652 (1957)
- 67 Heckel, G P, and Allen, W M, *Am J Obstet Gynecol* 35, 131 (1938)
- 68 Heide, A von der, *Munch med Wochschr* 58 1705 (1911)
- 69 Hinterthur, W, *Zuchtungskunde* 8, 55 (1933), *Animal Breed Abstr* 1, 24 (1933-1934)
- 70 Hisaw, F L, *Proc Soc Exptl Biol Med* 23, 601 (1926)
- 71 Hisaw, F L, and Zarrow, M A, *Proc Soc Exptl Biol Med* 69, 395 (1948)
- 72 Hisaw, F L, and Zarrow, M A, *Vitamins and Hormones* 8, 151 (1950)
- 73 Hooker, C W, and Forbes, T R, *Endocrinology* 41, 158 (1947)
- 74 Hooper, E C, *Proc Soc Exptl Biol Med* 31 1115 (1934)
- 75 Howell C E, and Rollins, W C, *J Animol Sci* 10, 789 (1951)
- 76 Huggett, A St G, *Conf on Gestation Trans 1st Conf, Princeton, New Jersey, 1954*, 53 (1955)
- 77 Inefchen, B, Dissertation, Univ Zurich, 1946, *Animal Breed Abstr* 16 967 (1948)
- 78 Jablonski, W J, and Vehrdo, J T, *Endocrinology* 61, 711 (1957)
- 79 Jasper, D E, Kendrick, J W, Holm, L W, and Clegg M T, unpublished observations
- 80 Jasper, D F, *Cornell Vet* 40 165 (1950)
- 81 Johnson L L, *Proc S Dakota Acad Sci* 24, 27 (1941)
- 82 Jordao L P, and Veiga, J S, *Rev Ind animal (Sao Paulo) [N.S.]* 1, 3 (1938), *Animal Breed Abstr* 7, 120 (1939)
- 83 Jordao, L P, and Veiga J S, *Rev Ind animal [N.S.]* 2 27 (1939), *Animal Breed Abstr* 8 128 (1940)
- 84 Jost A, *Cold Spring Harbor Symposia Quant Biol* 19 167 (1954)
- 85 Joubert D M, and Bonuma J C, *S African J Sci* 53 310 (1957)

7. Andrews, F. N., and McKenzie, F. F., *Missouri Univ. Agr. Exptl. Stat. Bull.* No. 329 (1941).
8. Asdell, S. A., "Mammalian Reproduction." Comstock, Ithaca, New York, 1946.
9. Asdell, S. A., *J. Agr. Sci.* 19, 382 (1929).
10. Balard, P., *Compt. rend. soc. biol.* 82, 1113 (1919).
11. Bard, P., *Psychosomat. Med.* 4, 171 (1942).
12. Basset, E. G., and Phillips, D. S. M., *Nature* 174, 1020 (1954).
13. Bell, C. H., and Robson, J. M., *Quart. J. Exptl. Physiol.* 27, 205 (1938).
14. Bonadonna, T., and Valerani, L., *Zootec. e vet.* 1, 129, 274 (1946). *Animal Breed Abstr.* 15, 174 (1947).
15. Bonsma, F. N., *Unio. Publ. Pretoria Ser. I Agr.* 48 (1939).
16. Bourgairel, R., and Ferrante, L., *Bull. fédération soc. gynéc. et. obstét. langue franç* 3, 239 (1951).
17. Brakel, W. J., Rife, D. C., and Salisbury, S. M., *J. Dairy Sci.* 35, 179 (1952).
18. Braude, R., and Walker, R. M., *J. Agr. Sci.* 39, 156 (1949).
19. Brewer, D. B., *J. Pathol. Bacteriol.* 73, 59 (1957).
20. Britton, J. W., and Howell, C. E., *J. Am. Vet. Med. Assoc.* 102, 427 (1943).
21. Brown, W. H., and Hirsch, E. F., *Am. J. Pathol.* 17, 731 (1941).
22. Buch, N. C., Tyler, W. J., and Casida, L. E., *J. Dairy Sci.* 38, 73 (1955).
23. Burris, M. J., and Blunn, C. T., *J. Animal Sci.* 11, 34 (1952).
24. Casida, L. E., and Venzke, W. G., *Proc. Am. Soc. Animal Production 29th Meeting.* 221 (1936).
25. Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.* 55, 167 (1934).
26. Chang, H. C., Lee, L. Y., Meng, C. W., and Wang, Y. K., *Proc. Soc. Exptl. Biol. Med.* 49, 380 (1942).
27. Chang, H. C., *Proc. Soc. Exptl. Biol. Med.* 32, 1001 (1935).
28. Clegg, M. T., unpublished observations, 1958.
29. Coburn, F. D., "Swine Husbandry." Orange Judd, Co., New York, 1919.
30. Cochran, R. L., and Meyer, R. K., *Proc. Soc. Exptl. Biol. Med.* 96, 155 (1957).
31. *Cold Spring Harbor Symposia Quant. Biol.* 19 (1954).
32. Cortez, E., *Rev. militar Remonta Vet.* 10, 149 (1952).
33. Csapo, A., *Am. J. Anat.* 98, 273 (1956).
34. De Boer, S., Dreyer, N. B., and Clark, A. J., *Arch. intern. pharmacodynamie* 30, 141 (1925-1926).
35. De Snoo, K., *Monatsschr. Geburtshilfe u. Gynäkol.* 57, 1 (1922).
36. Dey, F. L., Fisher, C., and Ranson, S. W., *Am. J. Obstet. Gynecol.* 42, 459 (1941).
37. Dicker, S. E., and Tyler, C. M., *J. Physiol. (London)* 121, 206 (1953).
38. Dicker, S. E., and Tyler, C. M., *J. Obstet. Gynaecol. Brit. Empire* 63, 690 (1956).
39. Dry, F. W., *New Zealand J. Agr.* 47, 386 (1933).
40. Edgar, D. G., *J. Endocrinol.* 10, 54 (1953).
41. Ehrenberg, R., and Liebenow, W., *Arch. ges. Physiol. Pflüger's* 201, 387 (1923).
42. Elkin, D. C., *J. Am. Med. Assoc.* 78, 27 (1922).
43. Evans, E. I., and Miller, F. W., *Am. J. Physiol.* 116, 44 (1936).
44. Fekete, K. von, *Endokrinologie* 7, 364 (1930).
45. Ferguson, J. K. W., *Surg. Gynecol. Obstet.* 73, 359 (1941).
46. Finerty, J. C., *Physiol. Recs.* 32, 277 (1952).
47. Fitzpatrick, R. J., in "The Neurohypophysis" (H. Heller, ed.), p. 203. Academic Press, New York, 1957.

- 48 Fleming, A M, *J Obstet Gynaecol Brit Empire* 35, 247 (1928)
- 49 Flexner, L B, Cowie, D B, Hellman, L M, Wilde, W S, and Vosburgh, G J, *Am J Obstet Gynaecol* 55, 469 (1958)
- 50 Forbes, T R, and Hooker, G W, *Endocrinology* 61, 281 (1957)
- 51 Fortgang, A, and Simpson, M E, *Proc Soc Exptl Biol Med* 84, 663 (1953)
- 52 Frank, R T, Bonham, C, and Gustavson, R G, *Am J Physiol* 74, 395 (1925)
- 53 Franklin, K J, and Winstone, N E, *Am J Physiol* 125, 43 (1954)
- 54 Gerlaugh, P, Kunkle, L E, and Rife, D G, *Ohio Agr Expt Sta Research Bull* 703 (1951)
- 55 Goltz, F, and Freusberg, A, *Arch ges Physiol Pfluger's* 9, 552 (1874)
- 56 Graham, E F, and Dracy, A E, *J Dairy Sci* 36, 772 (1953)
- 57 Gregory, P W, Mead, S W, and Regan, W M, *Portugaliae Acta Biol Sér A, R B Goldschmidt Vol* p 861 (1951)
- 58 Gunther, M, *Brit Med J* 1, 567 (1948)
- 59 Hall, K, *J Endocrinol* 15, 108 (1957)
- 60 Hallgren, W, *Nord Veterinarmed* 3, 1043 (1951), *Vet Bull Commonwealth Bur Animal Health* 22, 556 (1952), abstract
- 61 Hammond, J, and Marshall, F H A, *Wiss Ber Weltgeflugelkong* 1, 153 (1936)
- 62 Harris, G W, *Arch Gynaekol* 183, 35 (1953)
- 63 Haskins, A L, *Am J Obstet Gynecol* 67, 330 (1954)
- 64 Haterius, H O, and Ferguson, J K W, *Am J Physiol* 124, 314 (1938)
- 65 Hawker, R W, *J Endocrinol* 13, vP (1955)
- 66 Hawker, R W, and Robertson, P A, *Endocrinology* 60, 652 (1957)
- 67 Heckel, G P, and Allen, W M, *Am J Obstet Gynecol* 35, 131 (1938)
- 68 Heide, A von der, *Munch med Wochschr* 58, 1705 (1911)
- 69 Hüntherth, W, *Zuckungskunde* 8, 55 (1933), *Animal Breed Abstr* 1, 24 (1933-1934)
- 70 Hisaw, F L, *Proc Soc Exptl Biol Med* 23, 661 (1926)
- 71 Hisaw, F L, and Zarrow, M A, *Proc Soc Exptl Biol Med* 69, 395 (1948)
- 72 Hisaw, F L, and Zarrow, M A, *Vitamins and Hormones* 8, 151 (1950)
- 73 Hooker, C W, and Forbes, T R, *Endocrinology* 41, 158 (1947)
- 74 Hooper, E C, *Proc Soc Exptl Biol Med* 31, 1115 (1934)
- 75 Howell, C E, and Rollins, W C, *J Animal Sci* 10, 789 (1951)
- 76 Huggett, A St G, *Conf on Gestation Trans 1st Conf, Princeton, New Jersey, 1954*, 53 (1955)
- 77 Ineichen B, Dissertation, Univ Zurich, 1946, *Animal Breed Abstr* 16, 967 (1948)
- 78 Jablonski, W J, and Velardo, J T, *Endocrinology* 61, 741 (1957)
- 79 Jasper, D E, Kendrick, J W, Holm, L W, and Clegg M T, unpublished observations
- 80 Jasper, D E, *Cornell Vet* 40, 165 (1950)
- 81 Johnson, L F, *Proc S Dakota Acad Sci* 24, 27 (1944)
- 82 Jordão, L P, and Veiga, J S, *Rev ind animal (São Paulo) [N.S.]* 1, 3 (1938), *Animal Breed Abstr* 7, 120 (1939)
- 83 Jordão, L P, and Veiga, J S, *Rev ind animal [N.S.]* 2, 27 (1939), *Animal Breed Abstr* 8 125 (1940)
- 84 Jost, A *Cold Spring Harbor Symposia Quant Biol* 19 167 (1954)
- 85 Joubert D M, and Bonsma, J C, *S African J Sci* 53 340 (1957)

86. Karn, M. N., *Ann. Eugenics* 14, 44 (1947).
87. Keiffer, H., *Bull. acad. med. Belg.* 15, 581 (1935).
88. Kelly, G. L., *Anat. Record* 45, 225 (1930).
89. Kennedy, P. C., Kendrick, J. W., and Stormont, C., *Cornell Vet.* 47, 160 (1957).
90. Kenneth, J. R., *Tech. Commun. Imp. Bur. Animal Breed. Genet. No. 5*, (1953).
91. Kirkham, W. B., *Anat. Record* 11, 31 (1916).
92. Knapp, B., Lambert, V. W., and Black, W. H., *J. Agr. Sci.* 61, 227 (1940).
93. Knaus, N., "Periodic Fertility and Sterility in Woman." Wilhelm Maudrich, Vienna, 1934.
94. Knott, J. C., *J. Dairy Sci.* 15, 87 (1932).
95. Krizenecky, J., *Akad. Zemed.* 10, 351 (1935).
96. Kross, I., *Am. J. Obstet. Gynecol.* 11, 64 (1926).
97. Leonard, S. L., Hisaw, F. L., and Fevold, H. L., *Am. J. Physiol.* 100, 111 (1932).
98. Leopold, C., *Arch. Gynakol.* 11, 443 (1877).
99. Levin, L., Katzman, P., and Doisy, E. A., *Endocrinology* 15, 207 (1931).
100. McEntee, K., Roberts, S. J., and Sears, R. M., *Cornell Vet.* 42, 355 (1952).
101. McKeown, T., and MacMahon, B., *J. Endocrinol.* 13, 309 (1956).
102. Marder, S. N., and Money, W. L., *Endocrinology* 34, 115 (1944).
103. Marshall, F. H. A., and Peel, W. R., *J. Agr. Sci.* 3, 383 (1910).
104. Martin, E. A., *Hojas divulgadoras Agr. Madrid* 34 (23) (1942).
105. Martin, H., *Arch. Gynakol.* 183, 560 (1953).
106. Martynova, N. V., *Akusherstvo i Ginekol.* 9, 28 (1940).
107. Mauch, A., *Zücht Reihe B. Z. Tierzücht. Züchtungs-biol.* 39, 31 (1937).
108. Muhrer, M. E., Shippin, O. F., and Lasley, J. F., *J. Animal Sci.* 14, 250 (1955).
109. Neher, G. M., and Zarrow, M. X., *J. Endocrinol.* 11, 323 (1954).
110. Nelson, M. M., and Evans, H. M., *Endocrinology* 55, 543 (1954).
111. Nelson, W. O., Pfiffner, J. J., and Haterius, H. O., *Am. J. Physiol.* 91, 690 (1930).
112. Pearson, O. P., and Enders, R. K., *J. Exptl. Zool.* 95, 21 (1944).
113. Pencharz, R. I., and Long, J. A., *Am. J. Anat.* 53, 117 (1933).
114. Perry, J. S., *Vet. Record* 66, 706 (1954).
115. Pickard, J. N., *Proc. 4th World's Poultry Congr. London, England Sect. F* p. 901 (1930).
116. Pozo Lora, R., *Arch. Zootec.* 3, 265 (1954); *Biol. Abstr.* 30, 185 (1956).
117. Quinlan, J., Mayer, G. S., and Roux, L. L., *Rept. Director Vet. Serv. Animal Ind. Onderstepoort* 18, 831 (1932).
118. Rasbech, N. O., *Nord. Veterinarmed.* 2, 123 (1950).
119. Rasbech, N. O., *Nord. Veterinarmed.* 2, 655 (1950).
120. Rein, G., *Arch. ges. Physiol. Pflüger's* 23, 68 (1880).
121. Reynolds, S. R. M., *Am. J. Obstet. Gynecol.* 29, 630 (1935).
122. Reynolds, S. R. M., "Physiology of the Uterus," 2nd ed. Hoeber, New York, 1949.
123. Rife, D. C., Gerlaugh, P., Kunkle, L. E., Brandt, G., and Snyder, L., *J. Animal Sci.* 2, 50 (1943).
124. Roberts, S. J., "Veterinary Obstetrics and Genital Diseases." S. J. Roberts, Ithaca, New York, 1956.

- 125 Robson, J M, 'Recent Advances in Sex and Reproductive Physiology,' 3rd ed, Churchill, London, 1947
- 126 Robson, J M, *J Physiol (London)* 79, 139 (1933)
- 127 Robson, J M, and Schild, H O, *J Physiol (London)* 92, 1 (1938)
- 128 Robson, J M, *J Physiol (London)* 84, 121 (1935)
- 129 Rollins, W C, and Howell, C E, *J Animal Sci* 10, 797 (1951)
- 130 Rollins, W C, Laben, R C, and Mead, S W, *J Dairy Sci* 39, 1578 (1956)
- 131 Rosahn, P D, Greene, H S N, and Hu, C H, *Science* 79, 526 (1934)
- 132 Routh, A, *Trans Obstet Soc London* 39, 191 (1898)
- 133 Sauerbruch, F, and Heyde, M, *Munch med Wochschr* 57, 2617 (1910)
- 134 Short, R V, *Ciba Foundation Colloq Endocrinol* 11, 362 (1957)
- 135 Simonnet, H, Robey, M, and Paux, G, *Arch Soc Franc Biol Med* 31, 9 (1955)
- 136 Smith, G van S, Smith, O W, and Pincus, G, *Am J Physiol* 121, 98 (1938)
- 137 Smith, M G, *Bull Johns Hopkins Hosp* 49, 203 (1927)
- 138 Smith, P E, *Am J Physiol* 99, 345 (1932)
- 139 Smith, P E, *Anat Record* 94, 497 (1946)
- 140 Smith, P E, *Endocrinology* 55, 655 (1954)
- 141 Smythe, R H, *Vet Record* 66, 890 (1954)
- 142 Snyder, F F, *Bull Johns Hopkins Hosp* 54, 1 (1934)
- 143 Sorterup, E, *Norsk Vet Tidsskr* 45, 581 (1933)
- 144 Spencer, E, *J Roy Agr Soc Engl* 1, 165 (1840)
- 145 Spiegelberg O, *Lehrbuch der Geburtshilfe* 2 (1891)
- 146 Staffe, A, *Z Zucht Reihe B* 31, 79 (1934), *Animal Breed Abstr* 3, 126 (1935)
- 147 Steinetz, B C, Beach, V L, and Kroc, R L, *Endocrinology* 16, 271 (1957)
- 148 Stormont, C, Kendrick, J W, and Kennedy, P C, *Records Genet Soc Am* 25, 663 (1956)
- 149 Tassovatz, S, *Bull Soc Gynecol Obstet* 21, 74 (1932)
- 150 Terrill, C E, and Hazel, L N, *J Vet Research* 8, 66 (1947)
- 151 Thomson A M, and Thomson, W, *Brit J Nutrition* 2, 290 (1949)
- 152 Torda, C, *Proc Soc Exptl Biol Med* 51, 398 (1942)
- 153 Uppenborn, W Z *Zucht Reihe B Z Tierzucht Zuchtungs biol* 28, 1 (1933), *Animal Breed Abstr* 1, 158 (1933 1934)
- 154 Uren, A W, *Mich State Univ Agr Exptl Sta Tech Bull* No 144 (1935)
- 155 Villee, C A, *J Biol Chem* 205, 113 (1953)
- 156 Van Wagenen C, and Newton, W H, *Am J Physiol* 129, 485 (1940)
- 157 Von Oettingen, B, 'Horse Breeding in Theory and Practice' Sampson Low, Marston & Co, London, 1909
- 158 Wehefritz, E, *Arch Gynakol* 124, 511 (1925)
- 159 Wilson, W A, and Dudley, F J, *J Genet* 50, 384 (1952)
- 160 Williams, W L, 'Veterinary Obstetrics,' 4th ed W L Williams, Ithaca, New York, 1913
- 161 Wing H H, *Cornell Univ Agr Exptl Sta Bull* No 162 (1899)
- 162 Wishart, J, and Hammond, J, *J Agr Sci* 23, 463 (1933)
- 163 Woodbury, R, Abreu, B E, Torpin, R, and Fried, P H, *J Am Med Assoc* 128, 555 (1945)
- 164 Woodbury, R A, Ahlquist, R P, Abreu, B, Torpin, R, and Watson W G, *J Pharmacol Exptl Therap* 86, 359 (1940)

165. Woodbury, R. A., Hamilton, W. F., and Torpin, R., *Am. J. Physiol.* **121**, 640 (1938).
166. Zarrow, M. X., *Proc. Soc. Exptl. Biol. Med.* **66**, 488 (1947).
167. Zarrow, M. X., and Neher, G. M., *Endocrinology* **56**, 1 (1955).
168. Zarrow, M. X., Holmstrom, E. G., and Salhanick, H. A., *J. Clin. Endocrinol.* **15**, 22 (1948).
169. Zarrow, M. X., Neher, G. M., Sikes, D., Brennan, D. M., and Bullard, J. F., *Am. J. Obstet. Gynecol.* **72**, 260 (1956).
170. Zarrow, M. X., *Conf. on Gestation Trans. 3rd Conf., Princeton, New Jersey*, 1956, 17 (1957).

CHAPTER 16

Mammary Growth and Lactation

JOSEPH METTES

	<i>Page</i>
I Introduction	539
II Hormonal Requirements for Udder Growth	540
A Gonadal Hormones	540
B Anterior Pituitary Hormones	542
C Other Factors	545
III Hormonal Requirements for Lactation	546
A Anterior Hypophysis	547
1 Prolactin (Lactogenic Hormone)	547
2 ACTH and Adrenal Cortical Hormones	554
3 Other Hormones	554
B The Nervous System and Lactation	555
C The Mechanisms Controlling the Initiation of Lactation at Parturition	559
D Experimental Induction of Lactation in Farm Animals with Hormones	565
1 Methods of Hormone Treatment	566
2 Milk Yields and Lactation Curves	568
3 Effects on Reproductive Function and Health	577
E Increase of Established Lactation with Hormones	577
1 Pituitary Hormones	579
2 Thyroactive Substances	582
3 Estrogens	584
Acknowledgments	589
References	589

I INTRODUCTION

Mammary growth and lactation represent important phases of the reproductive cycle of mammals. Growth of the products of conception in the uterus and of the mammary glands occurs during gestation, this is followed by the initiation of labor and onset of milk flow at the end of pregnancy. Milk provides an essential and highly digestible form of nutrients for the young during a critical period after birth, just as the uterus provides a source of nutrients for the fetus before birth. These events, during and after gestation, are highly synchronized by the endocrine system, to a large extent, the same hormones which control growth and function of the uterus and its contents also control growth and function of the mammary glands.

The mammary glands are derived from the skin and are formed by

invagination of the ectoderm. Development begins during fetal life with a simple, branched, tubular gland which is transformed into a compound tubuloalveolar structure during gestation. Extensive growth does not usually begin until gestation and is largely completed by the end of the first two-thirds of pregnancy. After the onset of lactation at parturition, milk production rises for a relatively brief period and then gradually declines, and the lobuloalveolar system undergoes involution. These events are repeated after the next cycle of fertilization and pregnancy. The nervous system may not be essential for either mammary growth or initiation of lactation, but has an important role in the maintenance of milk secretion after parturition.

Most of our present knowledge of the factors governing mammary growth and lactation comes from studies made during the last 30 years in laboratory animals. Increasing attention has been given in recent years to the solution of lactational problems in farm animals, and considerable progress has been made in determining some of the experimental conditions necessary for optimal growth of the udder, initiation of lactation, and increase of established milk yields. These studies have been aided by the increased availability of hormones in relatively inexpensive forms. Emphasis will be given here to studies in dairy animals whenever possible, but there is little reason to suppose that endocrine control of mammary growth and lactation differs markedly among mammalian species except in an important quantitative sense.

II. HORMONAL REQUIREMENTS FOR UDDER GROWTH

The presence of an adequately developed udder is prerequisite to good lactational performance. A high correlation has been demonstrated between the amount of surface epithelium present in the goat udder during gestation and the subsequent volume of milk produced (32). A good correlation has also been reported (9, 138) between the total alveolar area, developed experimentally in the goat udder by estrogens or estrogen-progesterone combinations, and milk yield (Fig. 1).

A. Gonadal Hormones

The pioneer investigations of Turner and co-workers (156) established that estrogens are mainly responsible for duct growth, and estrogens and progesterone together are necessary for normal lobuloalveolar development. Estrogens alone, particularly in large doses, may induce considerable but usually abnormal growth of the lobuloalveolar system in a number of species. In the guinea pig, estrogens were reported to induce full lobuloalveolar growth, but recent findings are

not in agreement with this earlier work (10). In adrenalectomized guinea pigs, estrogens appear to induce only duct growth (72). Injections of estrogens into cattle and goats elicited mammary abnormalities, characterized by cystic alveoli and papillomatous outgrowths, whereas

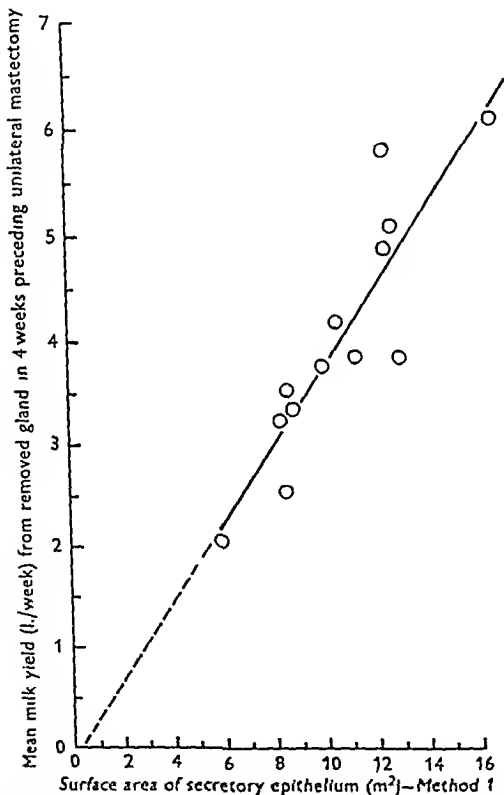


FIG. 1. Relation between surface area of secretory epithelium of goat udder developed by hexoestrol and progesterone, and milk yield (9).

combinations of estrogens and progesterone produced growth comparable to that seen in normal udders during gestation (9, 116, 151). These differences can be seen in Fig. 2.

The ratio of estrogen to progesterone and the absolute amount of each hormone present are important in determining the quantity and quality of mammary development. In the rabbit (140), maximum mammary growth occurs following injections of 24 to 96 μ g. of estrone and 1 mg. of progesterone daily (ratio of 1:11 to 1:42), while in the mouse, rat, and dog, optimal ratios are believed to be of the order of one part of estrogen to 1000 parts or more of progesterone (41).

The requirements for optimal udder growth in cattle and goats have not been adequately determined, and estrogens alone or combinations of estrogen and progesterone in ratios of 1:2 to 1:1000 have been employed with wide variations in total amounts of hormones given. In goats (32), injections of 1 mg. of hexestrol and 40 mg. of progesterone daily (ratio of 1:40) induced abnormal udder growth similar to that elicited by hexestrol alone; injections of 0.5 mg. of hexestrol and 70 mg. of progesterone daily (ratio of 1:140) resulted in normal udder development (9).

Benson *et al.* (10) recently demonstrated, in guinea pigs, that administration of the same ratios, but different doses of estrogen and progesterone, resulted in differences in mammary responses which varied from both a quantitative and qualitative point of view. They concluded that the absolute doses of the two hormones given are more important than their ratios. Inadequate amounts of either or both hormones may fail to elicit maximum udder development, and too much estrogen may inhibit mammary growth (61, 140).

Despite a few indications to the contrary, no consistent differences in milk yields have been reported in cattle and goats treated with combinations of estrogen and progesterone as compared to estrogens alone, although there is some suggestion of a more uniform response to the latter treatment (9). However, milk production may not be an accurate gauge of mammary development, since lactation can be initiated even in an incompletely developed mammary system. There can be little doubt that both estrogen and progesterone are essential for complete mammary growth.

B. Anterior Pituitary Hormones

As a result of observations that ovarian hormones do not stimulate mammary development in the absence of the anterior pituitary, Turner and co-workers (155, 156) postulated that their effects were mediated

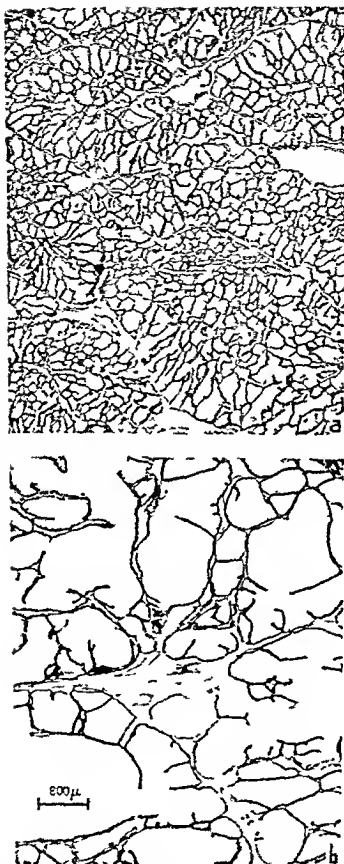


FIG. 2. (a) Normal alveolar tissue from goat given hexestrol and progesterone for 150 days. The alveoli are uniform and compact. (b) Abnormal alveolar tissue from goat given hexestrol alone for 150 days. Note extreme cystic condition (9).

through the anterior hypophysis. Estrogen was believed to stimulate the secretion of a pituitary duct-growth factor, mammogen I, and estrogen and progesterone together, of a lobuloalveolar factor, mammogen II. Later it was concluded that the two mammogens were the same, but distinct from any of the other established anterior pituitary hormones. Turner and others demonstrated that anterior pituitary extracts elicited some mammary growth activity when injected into intact or hypophysectomized mice or rats, and induced complete mammary development when administered together with ovarian hormones. Although the presence of a separate mammogenic factor, different from other anterior pituitary hormones, has not yet been established, the possibility can not be excluded that such a hormone is present in the pituitary.

Lyons *et al.* (91) reported that, in hypophysectomized, ovariectomized, virgin rats, mammary growth equal to that seen in early gestation could be induced by injecting prolactin, estrone, and progesterone. When the depression of body growth was partially prevented by injecting somatotropin (STH) in addition to the other three hormones, mammary development was induced equal to that seen in late pregnancy. Combinations of prolactin, estrogen, and progesterone appear to elicit better mammary growth responses than combinations of other anterior pituitary hormones with estrogen and progesterone in hypophysectomized rats (91). Prolactin injections are also effective in retarding mammary involution in lactating rats after their litters are removed (73), but this may be due to the maintenance of secretory function rather than to a growth action.

Despite the above experimental findings, it is difficult to assign any definite mammogenic role to prolactin during gestation. Assays of prolactin in the pituitary, blood, and urine of different species have shown that it is low during pregnancy, when maximum mammary growth occurs, and is high after parturition, when little or no mammary growth takes place. It is significant that mitotic activity in the mammary tissue is very high during gestation and virtually disappears after parturition (88, 134). The source of prolactin, the acidophil cells of the anterior pituitary, are low in number during gestation and increase markedly after parturition in the rat (101, 111). Also, mammary tissue from pregnant animals has relatively little ability to utilize prolactin, as indicated by the low rate of inactivation of prolactin when incubated *in vitro* together with mammary tissue from pregnant as compared to lactating rats (143, 144). The role of prolactin in mammary growth may be mainly "permissive" to the action of ovarian and other hormones, or there may be a factor closely associated with prolactin responsible for its

mammary growth effects Prolactin alone can not induce any notable degree of mammary growth in hypophysectomized animals

To what extent STH influences normal mammary growth is uncertain Whereas a combination of prolactin, STH, estrone, and progesterone induce full mammary development in hypophysectomized rats, the elimination of prolactin from this group results in only slight mammary growth (91, 92) STH has not been shown to appreciably retard mammary involution in rats and mice after removal of their suckling litters (76) STH alone has no mammary growth action in hypophysectomized animals

The ACTH-adrenal cortical mechanism does not appear to be essential but it can, nonetheless, influence mammary development (81, 154) Hohn (72) recently presented evidence that the ability of estrogens to induce lobuloalveolar development in the guinea pig depends on the presence of functional adrenal glands He found that administration of estrogens to adrenalectomized guinea pigs resulted only in duct growth, suggesting that hormones from the adrenal cortex similar to progesterone are necessary for full mammary growth Experimentally, administration of ACTH, deoxycorticosterone, aldosterone, cortisone, and hydrocortisone have elicited variable degrees of mammary growth in laboratory animals, and progesterone, estrogens, and male sex hormones have been isolated from the adrenal cortex The adrenal cortical hormones apparently are not as effective as the ovarian hormones in producing mammary development Thus, Mixner and Turner (116) reported that deoxycorticosterone acetate (DCA) was only about one-third as effective as progesterone in inducing lobuloalveolar growth in mice Flux (43) claimed that cortisone depressed the ability of estrogen to elicit duct growth in the mouse, but, in the rat, glucocorticoids alone or in combination with estrogens stimulated lobuloalveolar development (77, 142) Johnson and Meites (78, 80) reported that injections of cortisone acetate into lactating rats partially prevented involution of the mammary glands after removal of the litters

C Other Factors

The thyroid is not essential for normal mammary development, although it may modify the effects of other factors on mammary growth In the mouse, hypothyroidism reduces and mild hyperthyroidism enhances the mammary growth effects of ovarian hormones In the rat, the opposite results have been observed hypothyroidism increases, while hyperthyroidism decreases the effectiveness of ovarian hormones on mammary growth (116) This has been explained on the assumption

tion that the mouse is a relatively hypothyroid and the rat a relatively hyperthyroid species. Hypothyroidism in the bovine apparently results in subnormal udder development. Spielman *et al.* (147) reported decreased udder growth in cows thyroidectomized during gestation, and Petersen *et al.* (126) noted no visible udder growth in cows injected with diethylstilbestrol daily for 21 or 31 days following thyroidectomy. After feeding desiccated thyroid to the latter, injections of diethylstilbestrol resulted in udder growth and lactation. No histological examination was made of these udders.

The influence of the placenta in mammary development appears to vary in different species. Mammary regression was not observed in rats or mice hypophysectomized after mid-pregnancy, provided the placenta remained intact (91). Averill *et al.* (6) were able to maintain pregnancy and mammary growth in hypophysectomized rats before mid-pregnancy by implanting 12-day-old rat placentas, but this treatment was ineffective in the absence of the ovaries. The luteotropic activity demonstrated in the placenta of several species has not been shown to be identical with prolactin, despite the presence of minute amounts of prolactin in this tissue (30, 111). In some species, such as humans, monkeys, and horses, appreciable amounts of sex hormones may be secreted by the placenta and thereby supplement the action of the ovaries on mammary growth.

Androgens do not have any significant role in mammary development. Relatively large quantities of androgens are found in the feces of pregnant cows, but they appear to be metabolic products of progesterone (114). Experimentally, administration of relatively large doses of androgens are required to stimulate duct or lobuloalveolar development in laboratory animals (47).

III. HORMONAL REQUIREMENTS FOR LACTATION

The factors that control the *initiation* or *maintenance* of lactation, or are effective in experimental *galactopoiesis*, are not necessarily the same or of equal importance in each of these three processes. Thus, (a) estrogens can stimulate prolactin secretion and are believed to initiate lactation at parturition. After lactation is established, however, estrogens are not essential and in large amounts, may inhibit milk secretion. (b) The milking stimulus maintains secretion of several important pituitary hormones at high levels, and is therefore necessary for the maintenance of normal lactation. This neurohumoral mechanism is not present to initiate lactation before parturition. (c) Prolactin is essential for both initiation and maintenance of lactation, but is usually

a weak galactopoietic agent (d) In hypophysectomized animals, ACTH is necessary for initiation and maintenance of milk secretion, but has been reported to depress rather than to increase milk yields when injected into lactating cows (e) STH and TSH are necessary neither for initiation or maintenance of lactation, but exhibit greater ability to increase established milk yields in cattle than other hormones Other differences also occur in the control of these three processes

A Anterior Hypophysis

The anterior pituitary is essential for both initiation and maintenance of lactation Hypophysectomy in the cat (2) or dog (74, 90) during gestation prevents the onset of lactation after parturition In the rat or mouse hypophysectomized after mid pregnancy, a slight but transient lactation is observed for a few hours after parturition (60, 124) This has generally been attributed to the presence of small amounts of prolactin in the placenta, which may be stored rather than secreted by this tissue Hypophysectomy during established lactation invariably results in abrupt cessation of milk secretion

1 Prolactin (*Lactogenic Hormone*)

Two Swiss investigators, Stricker and Grueter (148), first demonstrated that anterior pituitary extracts contained a lactogenic factor which could initiate lactation in animals with developed mammary glands, an observation soon confirmed by other investigators Riddle and co workers (139) showed that this factor was identical with the anterior pituitary (AP) hormone which initiated crop milk secretion in pigeons The pigeon has since been the principal assay animal for prolactin (113)

Prolactin has been detected in the anterior pituitaries of many mammalian species, pigeons, and fowl, also in blood, urine, placental tissue, and cow manure (87, 111) Practically all workers agree that the acidophil cells of the anterior pituitary are the source of prolactin (30, 111) The term 'luteotropin' is sometimes used synonymously with, and even preferentially to, prolactin by many investigators, but the writer does not believe this is justified The lactation-inducing effect of prolactin is universal in mammals, whereas it has been shown to be definitely luteotropic only in the rat and mouse, and not in other species Satisfactory methods for quantitatively measuring prolactin in body fluids and tissues are not yet available However, assay methods for pituitary prolactin content are reliable, and although not necessarily indicative of secretory function, they have usually shown excellent agreement with the state of mammary secretory activity

The lactogenic hormone is believed to be the most important factor in the initiation and maintenance of lactation in intact animals with developed mammary glands. Other hormones are considered to be of secondary importance in the lactation process, despite the fact that another factor, ACTH, is needed in addition to prolactin to initiate milk secretion in hypophysectomized animals, or that some hormones may show greater galactopoietic activity than prolactin during established lactation. In intact animals with developed mammary glands, only an increase in prolactin is essential to initiate lactation.

The remarkable and specific action of prolactin on the mammary alveolar cells was first demonstrated by Lyons (89), who initiated lacta-

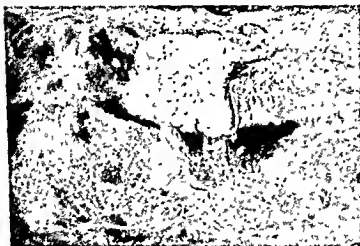


FIG. 3 Localized lactation induced by one injection of 10 I.U. of prolactin into a single duct of a teat of a rabbit.

tion in a localized segment of the mammary glands of rabbits by intraductal injections of small amounts of prolactin. This was confirmed by Meites and Turner (109), who showed, in addition, that only prolactin and no other hormone possessed this specific action (Fig. 3). These findings were corroborated by Bradley and Clarke (17). Although the British workers (46) previously stated that other factors, particularly ACTH, had an equal claim with prolactin to be considered as "lactogenic hormones," they now concede that "prolactin is the limiting factor in most experimental situations" (48).

a. Physiological Factors Influencing the Prolactin Content of the Pituitary. Mature females of all species studied except guinea pigs contain at least twice as much pituitary prolactin as mature males. Ovariectomy usually results in a decline, and administration of estrogens, in a marked increase in pituitary prolactin content. Rcece and

Turner (131) first demonstrated that estrogens could elicit an increase in pituitary prolactin content in rats, and this has been confirmed and extended to other species by Meites and Turner (111, 112). The effects of different levels of diethylstilbestrol on the prolactin content of the pituitary of male guinea pigs are shown in Fig. 4. Similar dose-response

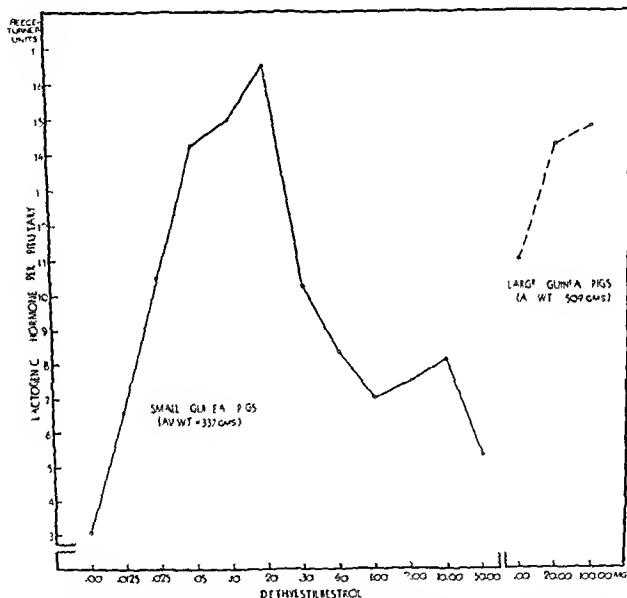


FIG. 4 Effects of graded doses of diethylstilbestrol on pituitary prolactin content of male guinea pigs

curves have been established in rats and rabbits. The greater effectiveness of small or moderate, as compared to large doses of estrogens in increasing pituitary prolactin, is also reflected in their more favorable effects on lactation. Thus, injections of 0.25 mg of diethylstilbestrol appear to be optimal for initiating lactation, but 1.0 mg daily is inhibitory to lactation in goats (115). Small or moderate doses of estrogens have also been shown to be more effective than larger doses in increasing the

number and secretory activity of the acidophils of the anterior pituitary (8, 168, 169).

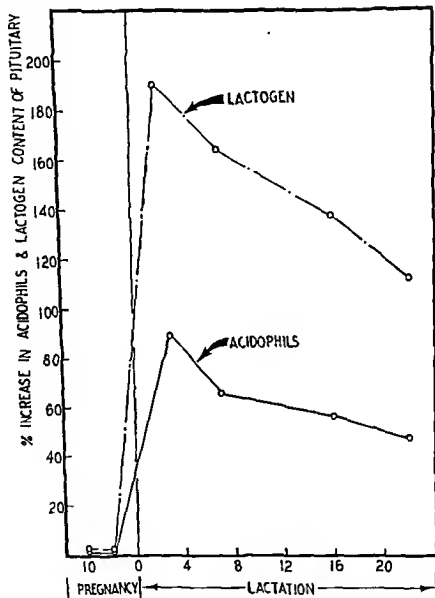


FIG. 5. Postpartum increases in prolactin content and acidophil cells in anterior pituitary of rats.

When estrogens are administered with progesterone in ratios of 1:1000-1:2000 into guinea pigs or rats, the ability of the former to elicit an increase in pituitary prolactin is considerably reduced. Some investigators have questioned whether such ratios are normally operative in the body (47), but recently it has been shown that these ratios are best for inducing mammary growth in rats, guinea pigs, and other

species (11, 162) It would appear that ratios of the two hormones which are optimal for inducing mammary growth are the most effective in inhibiting the stimulating action of estrogen on prolactin secretion Apparently, high levels of progesterone can inhibit other actions of estrogens, such as LH release, deciduoma response, etc, while other combinations of the two hormones may produce synergistic effects, i.e., mammary growth, progestational proliferation of the uterus, etc

During gestation, pituitary prolactin content remains low in most species studied, but increases rapidly after parturition In the rat, a parallel increase occurs in the acidophil cells of the anterior pituitary following parturition (Fig 5) At the end of pseudopregnancy, rabbits show no increase in pituitary prolactin content (108), and therefore do not come into milk secretion despite the presence of fully developed mammary glands Injections of prolactin promptly bring these animals into lactation Lactating rabbits bred on the first day of parturition

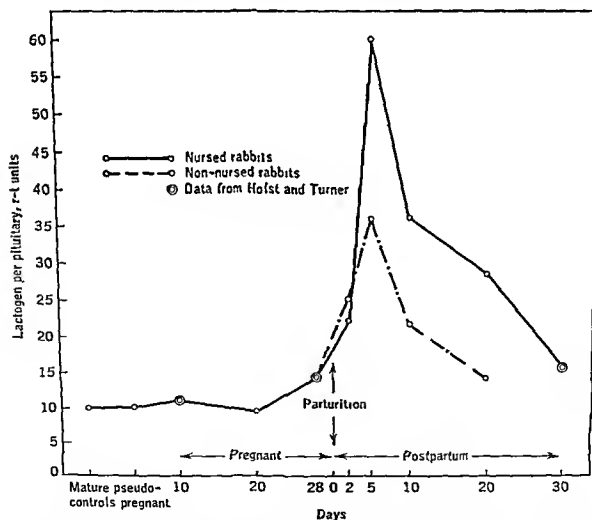


FIG 6 Relation of milking to postpartum changes in pituitary prolactin content of rabbits Note that initial increase as well as subsequent levels of prolactin were higher in suckled than in nonsuckled rabbits

show no greater decline in pituitary prolactin content during the ensuing gestation than open lactating rabbits (158). This is believed to demonstrate that the two ovarian hormones are not inhibitory to prolactin secretion during pregnancy, and that prolactin can be maintained at high levels by the milking stimulus.

The removal of suckling litters from lactating rabbits induces a more rapid decline in pituitary prolactin content than in suckled rabbits (Fig. 6). These results also show that maximum pituitary prolactin content is not attained after parturition in rabbits in the absence of the

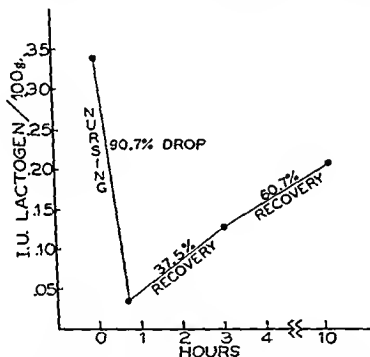


FIG. 7. Release and restoration of pituitary prolactin content in two strains of rats in response to nursing stimulus, after 10 hours isolation of mothers from litters. From (67).

nursing stimulus. When the suckling young of parturient rabbits were reduced to two each, pituitary prolactin content remained as high as in rabbits which were permitted to retain all their young (111). This suggests that a maximum intensity of the milking stimulus is not essential to maintain a high level of pituitary prolactin secretion. The immediate effect of the milking stimulus is to induce a quick release of pituitary prolactin, which is soon replenished during the interval between milking (Fig. 7). This is probably analogous to the immediate as opposed to the longer term effect of stress on ACTH secretion.

When given in large doses, progesterone or testosterone propionate induces small increases in pituitary prolactin; the latter may initiate a slight mammary secretion in the rat. Administration of ACTH, cortisone acetate, or hydrocortisone acetate (77) can also produce a moderate increase in the prolactin content of the pituitary and initiate secretion in the mammary glands of rats (Fig. 8). Deoxycorticosterone acetate does not increase pituitary prolactin in rats (159). Thyroidectomy or thiouracil administration have been reported to decrease pituitary prolactin content in rats (94, 110), but thyroxine injections apparently have no effect on prolactin content (111).

Petersen (125) suggested in 1942 that oxytocin stimulates increased secretion of prolactin by the anterior pituitary, and thus may be an important factor in the initiation and maintenance of lactation. British

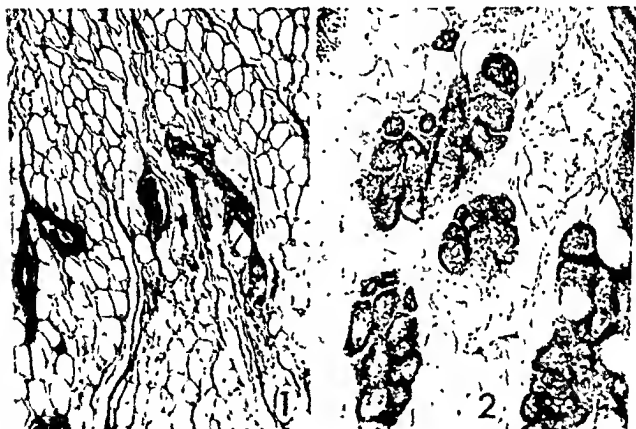


FIG. 8 (1) Mammary tissue from female rat injected with physiological saline. (2) Mammary tissue from female rat injected with 2 mg. cortisone acetate daily for 10 days. Note alveolar growth and secretion.

workers (11, 12) have recently expressed a similar viewpoint, based on observations that oxytocin injections partially inhibited the rate of mammary involution in parturient rats after removal of their litters, in a manner similar to that reported previously as a result of prolactin

injections (73). However, Meites and Turner (111) observed in 1942 that injections of posterior pituitary hormones failed to alter significantly the prolactin content of the pituitary of the rat, guinea pig, or rabbit. This has been confirmed more recently by Johnson and Meites (79) and Grosvenor and Turner (67). In addition, injections of oxytocin into pseudopregnant rabbits with well-developed mammary glands have not been found to initiate milk production (unpublished observations). There is thus no valid evidence that oxytocin influences prolactin secretion. The action of oxytocin in partially inhibiting mammary involution must be mediated through other mechanisms.

2. ACTH and Adrenal Cortical Hormones

The ACTH-adrenal cortical mechanism is believed to be second in importance to prolactin in the initiation and maintenance of lactation. In hypophysectomized guinea pigs or rats with developed mammary glands, ACTH or adrenal steroids are required, in addition to prolactin, to initiate lactation (63, 122). In rats hypophysectomized after parturition, prolactin alone has recently been reported to reinstate partial lactation, although a combination of prolactin and ACTH was somewhat more effective (27). Elias (39) recently demonstrated that prolactin and hydrocortisone were the minimal requirements necessary to maintain secretory activity *in vitro* in mammary tissue taken from mice in advanced pregnancy.

Adrenalectomy after mid-gestation does not prevent an increase in pituitary prolactin secretion or the onset of lactation after parturition, but milk secretion is of short duration (19, 104). Apparently, the hormones from the ovaries and perhaps the placenta partially substitute for the adrenal cortex during pregnancy. Adrenalectomy after parturition results in a gradual rather than in an abrupt cessation of lactation (49). Both the mineralo- and glucocorticoids have been shown to be essential for the reinstatement of full lactation after adrenalectomy in rats (29, 33, 62). There is some evidence that adrenal cortical activity is increased after parturition in the rat (129), and that the milking stimulus induces a release of ACTH from the pituitary (66, 152). In lactating rats, injections of ACTH and hydrocortisone prolong secretory activity and retard mammary involution after the litters are separated from their mothers (Fig. 9).

3. Other Hormones

Little work has been reported on the relation of the pancreas to lactation. In general, lactation can proceed in depancreatized bitches provided insulin is administered (123). Apparently no studies have

been made of the relation of diabetes to lactation in other species. Insulin increases lipogenesis from acetate and glucose in mammary slices from lactating rats (49), and may also participate in lactose synthesis. Injections of large doses of insulin decrease milk production in the cow (65), presumably by reducing the availability of glucose to the lactating udder.

Although milk synthesis involves heavy losses of Ca and P from the body, the relation of the parathyroids to lactation has received little attention. Campbell and Turner (23) observed that the parathyroid glands of rabbits were significantly enlarged during lactation as compared to the pregnant state, and that the degree of enlargement was directly related to the number of suckling young. Cowie and Folley (28) noted that lactation was severely depressed in rats following parathyroidectomy. In similarly operated rats, Munson (118) found that the Ca content of the milk showed no significant changes despite a reduction of blood Ca levels. When tetany in parathyroidectomized bitches was controlled during gestation by administration of calcium lactate or vitamin D, these animals were able to rear their litters (37). "Milk fever" in dairy cattle may be related to a deficiency of parathormone secretion at parturition. It has been successfully treated by prepartum administration of large doses of vitamin D (71), or by feeding a low calcium, high phosphorus diet (15) to stimulate parathyroid function. The influence of STH, TSH, and the thyroid will be considered elsewhere in relation to galactopoiesis in farm animals.

B. The Nervous System and Lactation

Selye (141) first demonstrated in the rat that the suckling stimulus is more important for the maintenance of lactation than removal of milk. He showed that if the galactophores were ligated so that the young could suckle but not obtain milk, secretory activity was extended and mammary involution was markedly retarded. Turner and Reincke (160) found that when milking was stopped on one-half of the udder of goats and the other half was milked regularly, secretion and retention of the lobuloalveolar system were considerably extended in the half-udder not milked. The constant placement of new litters to the nipples of virgin rats has been observed to elicit development and initiate milk secretion in the mammary glands (141).

The suckling stimulus has been reported to induce the release of prolactin (67, 132), oxytocin (42), and ACTH (66, 152) from the pituitary. Each of these hormones may prolong secretion and inhibit involution of the mammary glands of rats after removal of their litters.



FIG. 9 (Left) Control gland from rat 10 days after removal of young on 18th day postpartum.

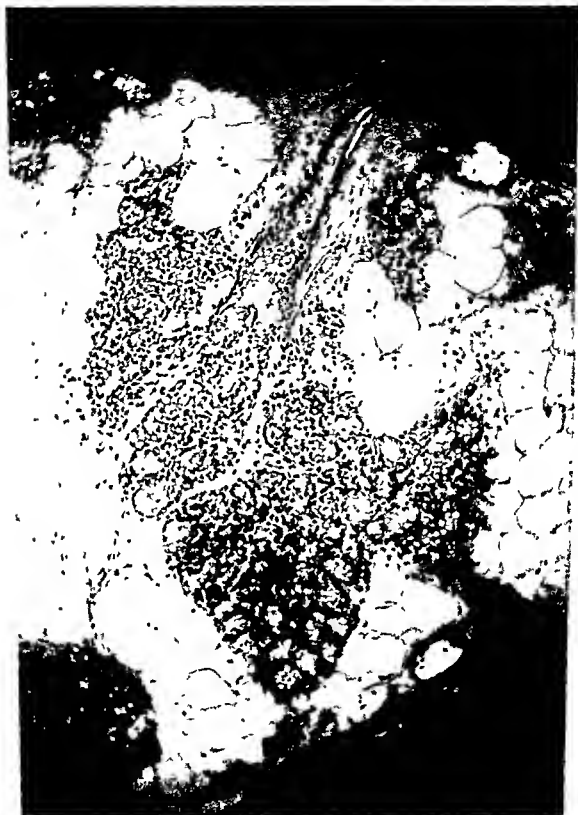


FIG. 9 (Right) Gland from rat 10 days after removal of young on 16th day postpartum and injected with 1.0 mg cortisone acetate daily. Note maintenance of alveolar structure and secretory activity.

The writer has recently observed that a combination of prolactin, oxytocin, and hydrocortisone was more effective in maintaining mammary secretory activity in rats after removal of their litters than any of these hormones given alone.

The amount of milk which accumulates in the cistern and larger ducts of the udder of cows and goats between milking is only a small portion of the total amount present. The greater part is held in the alveolar lumina and in the smaller ducts, and can be released only by the suckling or milking stimulus. This is believed to produce a release of oxytocin, which in turn brings about reflex contraction of the myo-epithelial cells surrounding the alveoli. The presence of the latter cells was first demonstrated by Richardson (137). The "squeezing" of the alveolar lumina results in increasing milk pressure, at which time the milk can be most easily evacuated from the udder. The milk discharge reflex can be conditioned by certain visual, auditory, olfactory, or other stimuli, just like other reflex mechanisms.

Gaines (59) first suggested that milk ejection is due to reflex contraction of smooth muscle elements in the udder in response to the milking stimulus, and demonstrated that injections of posterior pituitary extracts into an anesthetized bitch enabled the pups to obtain milk. However, he did not assign a physiological role to the posterior pituitary. Turner and Slaughter (161) first postulated that milk ejection is mediated through the posterior pituitary, and Ely and Petersen (42) presented in concrete form the currently accepted theory, namely that milk "let-down" is due to release of oxytocin and milk "hold-up" to release of epinephrine.

Petersen and Ludwick (127) demonstrated the presence of a milk discharge factor in the blood of a cow which had been milked, by passing this blood through the perfusion medium of an isolated udder, causing ejection of milk. *Blood from a cow which had not been milked* was without effect on milk ejection. Other workers have failed to demonstrate consistent alterations in the oxytocin content of the posterior pituitary or blood following the milking stimulus in the goat, cow, and woman (31, 70, 165), but this may be due to limitations in extraction and assay techniques.

Ely and Petersen (42) showed that injections of epinephrine prevented milk ejection in the cow, an observation confirmed in the sow (18) and rabbit (34), and suggested that the sympathetic nervous system was involved in inhibition of milk discharge resulting from fright or other stresses. Cross (34) demonstrated that stimulation of the posterior hypothalamus in the anesthetized rabbit inhibited milk ejection.

tion, similarly to epinephrine, and that this inhibition was abolished after adrenalectomy. He concluded that the principal factor involved in the peripheral block to circulating oxytocin was constriction of the mammary blood vessels.

Definite evidence for the role of the neurohypophysis in milk ejection was provided from experiments by Cross and Harris (35). They demonstrated that milk ejection could be elicited in the anesthetized rabbit by electrical stimulation of the supraoptico-hypophysial tract. Andersson (3) similarly observed milk ejection in lactating ewes and goats, following electrical stimulation of centers in or near the supraoptic nucleus of the hypothalamus. Studies in the rat indicate that oxytocin may be produced in the neural stalk rather than in the posterior lobe (31), since posterior lobectomy interferes with milk ejection during a concurrent lactation, but not after lactation is reinitiated by a subsequent gestation.

The evacuation of milk from the udder, with a resultant reduction in intramammary pressure, is apparently not a major factor in the maintenance of lactation. Recent experiments in cows have shown that intramammary pressure does not significantly affect the rate of milk secretion until about 16 to 20 hours after milking (40, 165).

C. The Mechanisms Controlling the Initiation of Lactation at Parturition

A number of theories, in agreement in some of their aspects and contradictory in others, have been presented to explain the absence of copious lactation during gestation and its onset at about the time of parturition. This phenomenon is not yet completely understood because of insufficient knowledge of the secretion rates of hormones controlling mammary growth and lactation during these periods. The two theories that have received the greatest attention are based on interactions among the pituitary, ovaries, and mammary gland.

In a series of experiments in guinea pigs, Nelson (120) noted that: (a) estrogens inhibited established lactation, an observation repeatedly confirmed by other workers, and (11) when estrogens and prolactin were injected together, lactation did not begin unless the estrogen injections were terminated. On the basis of these findings, the theory was advanced that during gestation estrogen suppressed secretion of prolactin by the anterior pituitary and also inhibited the mammary response to prolactin. The principal emphasis was placed on the inhibitory effects of estrogen on prolactin secretion. With the removal of estrogen in-

hibition at the end of gestation, prolactin presumably was secreted in sufficient amounts to initiate lactation.

This theory was challenged by Meites and Turner (105, 106, 107), mainly because: (a) estrogens were shown to increase rather than decrease the prolactin content of the pituitary and blood; (b) estrogens could initiate as well as inhibit lactation in a large variety of laboratory and farm animals; (c) lactation could continue during a concurrent pregnancy, despite the presence of high levels of estrogens; and (d) under appropriate conditions, lactation could be initiated by injecting prolactin into pregnant rabbits, when the mammary glands were actively growing, without disturbing gestation (109). Later, Nelson (121) emphasized that his theory applied only to the guinea pig and not necessarily to other species.

Estrogens, particularly in large doses, can depress established lactation in a number of species, including cows and goats (111, 112). The minimal amount of diethylstilbestrol necessary to inhibit established lactation in goats was reported to be at least 1.0 mg. daily (115), or four times as much as needed to initiate lactation in this species. Larger doses depress lactation more effectively, but the inhibition disappears after the injections are discontinued (Fig. 10). Large doses of estrogens may inhibit established lactation by decreasing thyroid function, depressing appetite, increasing adrenal cortical function, increasing nervousness, or by other means (49, 111, 112).

Meites and Turner (105, 106, 107) assigned a key role to estrogen as the chief stimulator of prolactin secretion, and as the indirect initiator of milk secretion at parturition. This was therefore diametrically opposed to the inhibitory role assigned to estrogen by Nelson. Indeed, as early as 1931, Turner and Gardner (157) first set forth the view that estrogen stimulates prolactin secretion and initiates lactation, i.e., "When the estrus-producing hormone increases, a concentration is reached at about the time of parturition, which activates the pituitary to secrete a lactation stimulating hormone in amounts sufficient to cause the initiation of milk secretion." Meites and Turner hypothesized (105, 106, 107) that during gestation prolactin secretion remained too low to induce abundant lactation because of the predominance of progesterone over estrogen, preventing the latter from increasing prolactin secretion. Toward the end of gestation, a shift of hormone balance in favor of estrogen was believed to result in increased prolactin secretion and initiation of lactation.

In recent years, the writer found it necessary to revise the above theory, without negating any of its original tenets, by including a role

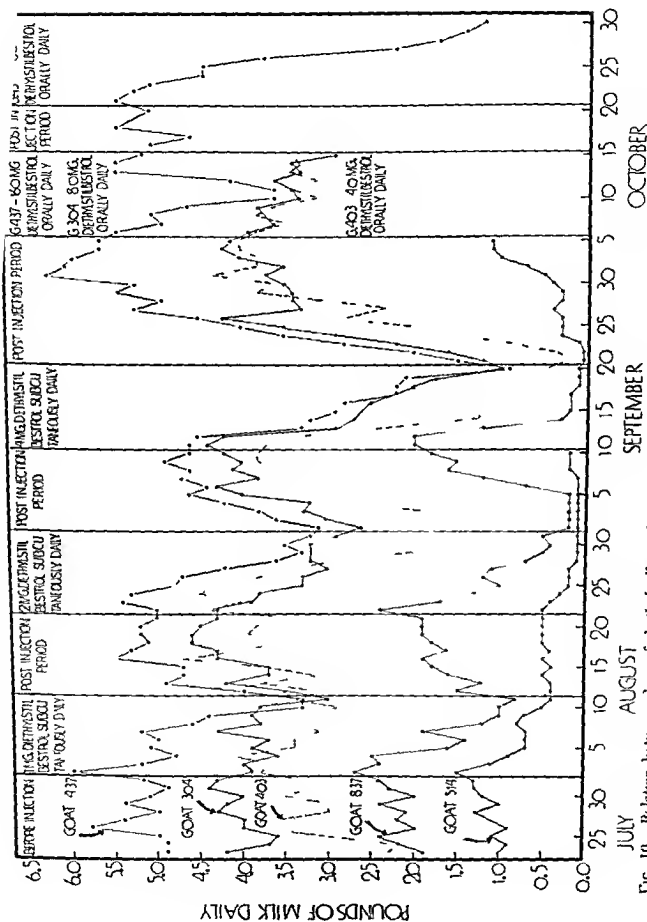


Fig. 10 Relation between dose of diethylstilboestrol injected and inhibition of milk production in 5 multiparous goats

for the relative antagonism between mammary growth and lactation now believed to be operating during gestation (95, 97, 102, 103). The idea that mammary growth was inhibitory to lactation was proposed many years ago (88), but was not widely accepted as valid for several reasons. Thus, (a) lactation and mammary growth proceed simultaneously in many species in which pregnancy occurs after parturition; (b) estrogen administration can at one and the same time induce mammary growth and initiate lactation; (c) prolactin, which initiates and helps maintain lactation, has been reported to produce some mam-



FIG. 11. Mammary growth versus secretion. The ovariectomized rabbit above received 0.096 mg. estrone and 1.0 mg progesterone daily for 25 days, followed by 2.0 mg. daily of prolactin for 10 days. Ovariectomized rabbit below treated similarly except that the two steroids were given for entire 35 days of experiment.

mary growth in intact animals (89, 91). This latter, however, may be mediated by some factor closely associated with prolactin rather than with prolactin itself.

It was not appreciated until recently that an antagonism between mammary growth and lactation does exist, but is *relative* rather than *absolute*. The absence or presence of lactation is believed to depend on the balance between the levels of prolactin and other factors operating to promote lactation, on the one hand, and the levels of estrogen and progesterone and other factors operating to promote mammary growth, on the other hand.

When estrone and progesterone were injected in doses and at a ratio

known to promote optimal mammary growth in rabbits, 0.096 and 1.0 mg., respectively, injections of a moderate dose of prolactin, 2 mg. daily, failed to initiate lactation if treatment with the two steroids was continued (Figs. 11 and 12). When larger doses of prolactin, 4 to 8 mg. daily, were injected into these animals, some lactation was initiated despite continued administration of the same doses of the two steroids. When the same ratio of the two steroid hormones was given, but in only half or quarter doses, even 2 mg. daily of prolactin was able to initiate



FIG. 12 Radioautographs of growing (left) and regressing (right) mammary glands from rabbits given a single intravenous injection of 50 μ g P^{32} per kilo of body weight and killed 4 hours later. Growth was maintained for 35 days by daily injections of estrone and progesterone, regression occurred when the two steroids were given for 25 days and then stopped for 10 days. The rabbits were killed on the 35th day and the mammary glands were dried and exposed to X-ray film for 72 hours. The growing gland did not respond to prolactin but the involuting gland showed a marked response (see Fig. 11).

lactation. Progesterone alone did not inhibit and estrone alone only slightly reduced the mammary response to prolactin.

In vitro studies also suggest that the growth action of the two ovarian hormones on the mammary gland make it relatively unresponsive to prolactin stimulation. Lactating mammary tissue from rats 4 days after parturition inactivated about eight times as much prolactin as an equiva-

lent amount of mammary tissue from rats in mid-gestation (143, 144). Respiratory studies by Folley (48) appear to be in agreement with these observations, since incubation of prolactin with mammary tissue from lactating rats increased the rate of net gas evolution, whereas no change occurred when mammary tissue from pregnant rats was incubated with prolactin. Sgouris and Meites (unpublished observations) also noted that prolactin increased oxygen consumption in mammary slices from parturient rats but not from rats in mid-pregnancy. The depression of established lactation which results from administering large doses of estrogens or estrogen-progesterone combinations may be induced partially by mammary growth stimulation.

The revised Meites-Turner theory of lactation can now be stated as follows: (a) In a pregnant, nonlactating animal, estrogen and progesterone levels promote full mammary growth, rendering the udder relatively insensitive to prolactin stimulation. (b) Progesterone during pregnancy prevents any notable stimulation by estrogen of prolactin secretion, leaving it at too low a level to initiate lactation. (c) At the end of pregnancy, there is a marked decline in both estrogen and progesterone, leaving the mammary gland receptive to prolactin stimulation, and at the same time sufficient estrogen is still present to increase pituitary prolactin secretion and initiate lactation. There is considerable evidence that estrogenic activity is increased at about the time of parturition (111). This theory is believed to be in accord with most experimental observations.

Lactation can proceed in parturient animals which subsequently become pregnant because: (a) relatively high levels of prolactin and other hormones favorable to lactation are secreted as a result of the milking stimulus, (b) estrogens and progesterone are secreted at much lower levels during early than in late pregnancy, and therefore the rate of mammary growth does not appreciably antagonize the action of prolactin and other factors favorable to lactation until the latter part of gestation. Fetal growth during the latter part of gestation may also partially account for the decline in lactation. This is believed to explain why dairy cows which are simultaneously lactating and pregnant, show no pronounced drop in milk yield until after the fifth month of gestation, following which time the decline is about 20% greater than in open cows (156).

This theory has been challenged by Folley and co-workers (30, 52), mainly because of (a) the interpretation placed on pituitary prolactin assays as an index of actual secretion rate, and (b) the emphasis placed on prolactin as the principal factor in the initiation of lactation. No

evidence has yet been presented by these or other workers which is in basic conflict with the conclusions drawn from our pituitary assays. Attention has already been given to the good agreement between pituitary prolactin content and the secretory state of the mammary gland. The emphasis on prolactin rather than on a "lactogenic complex," as the crucial factor in initiation of lactation in *intact* animals with developed mammary glands, is believed to be justified, since only prolactin administration is needed to induce lactation in such animals. Despite the exceptions noted above, the British workers have recently accepted the main tenets of our theory of lactation (30, 49).

D. Experimental Induction of Lactation in Farm Animals with Hormones

Many dairy animals with good milk-producing potential are discarded from herds each year because of breeding failures. Some of these animals could be profitably maintained on farms if suitable hormone treatments could be devised for bringing them into milk production equal or nearly equal in volume to that attained by animals after gestation. These hormone treatments may also produce other benefits: (a) some animals may be successfully bred after hormone administration is concluded and (b) a moderate or considerable increase in body weight and efficiency of feed utilization may result from the hormone treatment.

The experimental methods currently employed to induce lactation in dairy cattle and goats are based on use of estrogens alone or estrogen-progesterone combinations. Administration of estrogens elicits some udder development, even though it is incomplete and histologically abnormal in character. Estrogens also usually initiate precocious lactation, often within a few weeks after hormone administration begins. Other undesirable results which may ensue from use of estrogens alone are nymphomaniac behavior and distortion of the rump in cattle. When given in proper doses and ratios, combinations of estrogen and progesterone appear to produce more normal and complete udder development, without inducing precocious milk secretion, and may completely prevent the appearance of nymphomaniac symptoms. Subsequent administration of estrogens alone for a brief period usually results in initiation of lactation.

The milk from hormonally induced lactations in cattle and goats undergoes about the same changes as after normal parturition. It is initially colostrals in nature and then changes rapidly to milk of normal composition (51, 93). No significant amounts of hormones have been

detected in the milk of animals induced to lactate by estrogens and/or progesterone, and such milk is safe for human consumption.

Estrogens were first reported to initiate lactation in rabbits (58, 157) and rats (131, 132). Initiation of lactation in cattle and goats with estrogens was first reported independently by investigators in the United States (85, 86, 164) and England (50, 51, 54, 68). This has been widely confirmed and a number of improvements have been made in the methods and techniques employed earlier. However, milk yields in the majority of treated animals have averaged considerably less than those of animals following parturition, and the response has varied greatly among individual animals.

Knowledge of the actual hormone secretion levels during gestation and at parturition in domestic animals would undoubtedly be of great value in formulating better procedures than are currently available. The problem of controlling individual variability in response is more difficult. These variations may be caused by dissimilarities in initial hormone secretion rates, changes in endocrine balance (particularly the ovaries and anterior pituitary) as the result of hormone administration, differences in responsiveness of the udder, age, number of previous pregnancies, hereditary potential for milk production, weight, breed, nutritional status, and other causes. Methods have not yet been devised which will overcome most or all of these variables and ensure a satisfactory milk response in treated animals, but there is reason to believe that this can ultimately be achieved.

1. *Methods of Hormone Treatment*

Estrogens or estrogen-progesterone combinations have been administered to cattle, goats, and sheep by subcutaneous implantation of tablets or pellets, subcutaneous injections of solutions or macrocrystalline suspensions, feeding, inunction of the udder, and, in one report in goats (64) by intramammary injections through the teat canal. Neither oral administration nor udder inunction has proved to be very effective for inducing lactation, although the oral route would offer a number of obvious advantages in treating farm animals.

Relatively little relationship has been demonstrated between the amounts or ratios of hormones employed thus far and the lactational response in animals. However, it appears reasonable to assume that certain minimal amounts of hormones must be absorbed to ensure adequate udder development and milk production. A relationship between the amounts of hormones absorbed and milk yield is suggested in the results shown in Table I. It can be seen that the three animals which

TABLE I
RELATION BETWEEN HORMONAL TREATMENT AND MILK YIELD IN DAIRY CATTLE

Description of animal	Hormones implanted	Amount given (g.)	Amount absorbed (g.)	Duration of implant (days)	Peak daily milk prod. (lb.)	12 month	
						Milk (lb.)	Fat (lb.)
Guernsey heifer (J 1)	Prog	4.0	0.97	108	25.4 ^a	0.081	323
	Stilb.	2.0	0.52		29.2 ^b		
Guernsey heifer (J-2)	Prog	4.0	1.04	88	24.7 ^a	0.622	331
	Stilb.	2.0	0.78	168	29.8 ^b		
Guernsey heifer (J 3)	Prog	3.0	0.99	112	25.9	0.532	369
	Stilb.	0.6	0.39				
	Stilb. ^c	1.2	0.22	36			
Guernsey cow, 4 years old	Prog	3.0	0.50	90	3.5	—	—
	Stilb.	0.3					
Holstein cow (Mabel)	Prog	3.0	1.54	124	80.0	11,330	426
	Stilb.	0.1					
	Stilb. ^c	1.5	0.37	24			
Holstein cow (Julia)	Prog	3.0	1.10	121	45.0	7,760	309
	Stilb.	0.1					
	Stilb. ^c	1.5	0.44	34			
Holstein cow (Schert's)	Prog	3.0	3.00	120	56.0	11,930	466
	Stilb.	0.1	82				
	Stilb. ^c	1.5	25	30			
Holstein cow, 11 years old	Prog	3.0	0.08	120	3.0	—	—
	Stilb.	0.3					
	Stilb. ^c	1.5	0.02	30			
Brown Swiss cow, 5 years old	Prog	3.0	0.93	120	16.0	—	—
	Stilb.	0.3					
	Stilb. ^c	1.5	0.00	30			

^a After progesterone stilbestrol treatment only
Brackets indicate that hormones were given together

^b After thyroprotein feeding
^c Stilb. given after Prog-Stilb. treatment

absorbed the most hormones produced greater milk yields than the cattle which absorbed the least amounts of hormones. These results are not strictly comparable because of differences in breed, age, and treatment.

Benson *et al.* (9) questioned the need for administering a "triggering" dose of estrogen following prior treatment with both estrogen and progesterone in goats, since milk yields in goats given only the two hormones together did not differ significantly from yields in goats which were subsequently given a "triggering" dose of estrogen. However, examination of their data reveals that a high estrogen to progesterone dosage was used, giving mainly the effects of estrogen and hence obviating the need for further estrogen treatment. Meites *et al.* (100), Hancock *et al.* (69), and Turner *et al.* (162) all noted that when cattle were given relatively wide estrogen to progesterone ratios, filling of the udder with secretion did not usually occur until subsequent administration of additional estrogen.

2. Milk Yield and Lactation Curves

All breeds of cattle, goats, and sheep treated thus far appear to respond to hormone stimulation, and generally animals from good milk-producing progeny give higher milk yields than animals of poor milk-producing ancestry. Freemartins respond poorly to hormone administration (162). Most workers find that heifers respond more readily than cows, although total milk yields may be greater in the latter animals. There is some evidence that older goats produce more milk in response to hormone treatments than younger goats (119).

Daily milk yields in cattle have ranged from a few ounces per day to as much as 80 pounds daily in one Holstein cow, and in goats from a few milliliters daily to as much as four pounds per day. Some animals require a long latent period to come into lactation, while others respond quickly; some reach peak production after about the same interval as normal parturient animals, whereas others begin slowly and require a relatively long period to reach peak production. The duration of lactation in successfully treated animals appears to be about the same as in normal parturient animals.

An analysis of the highest daily milk yields in 142 heifers and cows treated with estrogen implants by Hammond and Day (68), shows that 86% of the animals produced 20 pounds or less (a few with no milk); 13.6%, between 21 to 30 pounds; and 1.4%, between 31 to 35 pounds. This represents the biggest lactation experiment yet reported with estrogens in cattle. In work reported by Marshall *et al.* (93), the lactation records of 13 diethylstilbestrol-injected Jersey heifers

and cows were compared with the lactation curves of 17 normal Jerseys after first calving. The results (Fig. 13) show lower yields in the treated than in the control animals, and slightly higher levels of production when injections of diethylstilbestrol were continued into lactation.

The response to combinations of estrogen and progesterone, whether treated subsequently with estrogens or not, has also shown considerable variation. Two nonbreeding Guernsey heifers were each implanted with pellets containing 2 gm. of diethylstilbestrol and 4 gm. of progesterone (1:2 ratio), with the intention of leaving these hormones

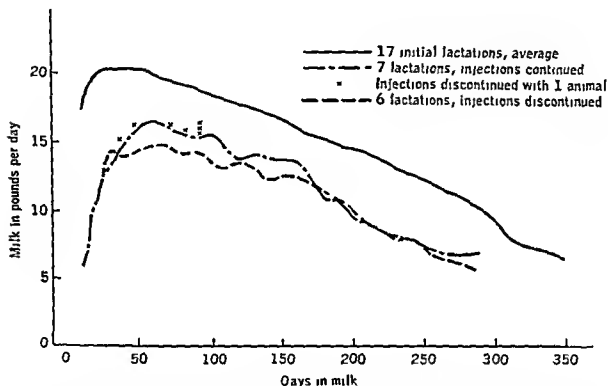


FIG 13 Average milk yields in first lactation after injection of stilbestrol-in-oil, with injections continued into the lactation period, with injections discontinued, and lactations following normal calvings without administration of stilbestrol. From (93)

in situ for 3 months (101). It was necessary to begin milking one heifer on the 64th day after implantation because her udder became filled with secretion, but no appreciable amount of milk could be obtained from the other heifer until after removal of the hormone pellets 96 days after implantation (Fig. 14). The large dose of estrogen given to these two animals obviated any need for additional estrogen after pellet removal.

Hancock *et al.* (69) injected one of each pair of identical twins with 1:1000 ratio of diethylstilbestrol to progesterone for a period of 5 months, followed by injections of diethylstilbestrol alone for another 30 days. Twice-daily milking was begun when the normal control mates calved. Milk production was 10.8, 83.7, and 106.9% of the yield attained by

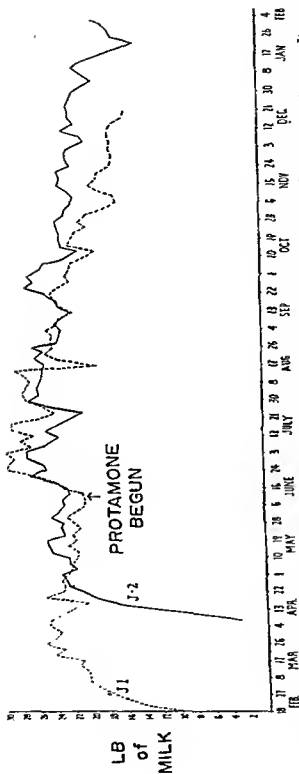


FIG. 11. Lactation curves of two Guernsey heifers implanted with 2 g. diethylstilbestrol and 4 g. progesterone. It was necessary to begin milking J-1 before J-2 because her udder became distended with milk. Thyroprotein (Protomone) was fed at a level of 15 g. daily and increased expected milk yields by about 20%.

the three lowest producers, and 41.9, 49.1, 61.1, and 72.5% of the yield of the four highest producers. Turner *et al* (162) similarly injected 10 heifers with 100 μ g of estradiol benzoate and 100 mg of progesterone (ratio of 1:1000) for 6 months, followed by injections of 3 mg of the estrogen daily for 14 days. These animals showed an average maximum

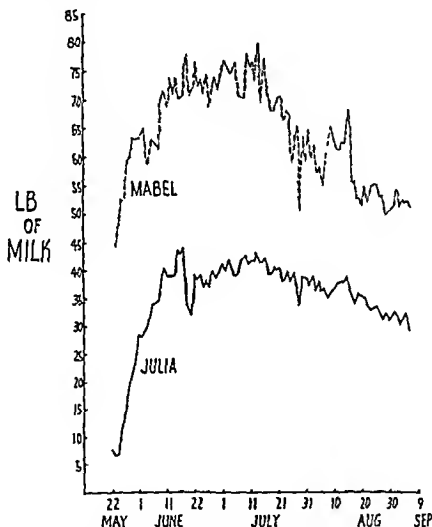


FIG. 15. Milk production curves for first 109 days of lactation in 2 Holstein cows implanted with 100 mg diethylstilbestrol and 3 g progesterone, followed 90 days later by 15 g diethylstilbestrol. Implants were removed at end of 120 days and milking was begun.

daily production of 23 pounds of milk, which the authors believed approximated the expected yields if these heifers had calved normally.

The best daily and total milk yields during a 10 month period of lactation initiated by hormone treatment were reported by Meites *et al* (100, 136) in 3 Holstein cows. These 3- to 4-year old animals had each calved once previously and failed to breed subsequently. They were each implanted with a total of 100 mg of diethylstilbestrol and 3.0 gm of progesterone in pellet form (1:30 ratio). At the end of 3 months, the

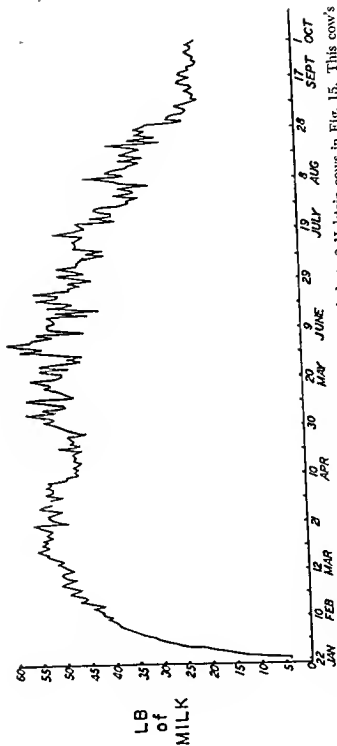


FIG. 16. Lactation curve of Holstein cow (Scherer's) treated similarly to 2 Holstein cows in Fig. 15. This cow's 10-month production was outstanding.

pellets were removed and a second implant was made of 1.5 gm. of diethylstilbestrol. The udders began to fill with secretion after the second implant was made and milking was begun a month later, at which time the diethylstilbestrol residue was removed. Their lactation curves are shown in Figs. 15 and 16 and photographs of two of the animals appear in Figs. 17 and 18. Their 10-month production as a result of hormone treatment compared as follows with their previous 10-month production after normal calving (DHIA records): Mable, 11,330 pounds



FIG. 17. View of Holstein cow (Mabel) when producing 75-80 pounds of milk daily as result of hormone implantation

of milk and 420 pounds of fat, compared to 366 pounds of fat, Julia, 7,760 pounds of milk and 309 pounds of fat, compared to 420 pounds of fat; Scherer's Holstein, 11,930 pounds of milk and 166 pounds of fat, compared to 13,397 pounds of milk and 481 pounds of fat. The highest daily yield in pounds for each of the 3 cows was as follows: Mable, 80, Julia, 45, Scherer's Holstein, 56. The majority of some 10 animals similarly treated produced less milk than the average of normal parturient cows (see Table I).

Milk production in goats resulting from hormone administration has also shown great variation, and has been below average in most cases

Figure 19 shows the difference in response of 5 virgin yearling goats which had been induced to lactate by subcutaneous injections of 0.25 mg. of diethylstilbestrol daily, a dose presumably optimal for initiating milk secretion in these animals (115). Better responses were obtained in 2 goats fed a combination of 60 to 75 mg. of a dimethyl ether of diethylstilbestrol and 1.5 to 1.75 gm. of thyroprotein daily following a long latent period (Fig. 20). Sykes (149) reported that of 45 yearling does



FIG. 18. View of udder of Holstein (Scherer's) when producing 56 pounds per day

injected with diethylstilbestrol, 11 did not produce measurable quantities of milk; 9 produced a total of less than 50 pounds; 10, between 50 and 100 pounds; and 12, between 300 to 500 pounds. Nellor and Reineke (119) injected goats with diethylstilbestrol and progesterone together for 9 to 11 weeks, followed by diethylstilbestrol alone for an additional 2 weeks. Milk yields ranged from 20.3 to 86.3% of that produced by these does during a subsequent lactation after pregnancy. Eaton *et al.* (38) observed a highly significant correlation between the yields induced in young goats by injections of diethylstilbestrol and the subsequent yields from the first natural lactation.

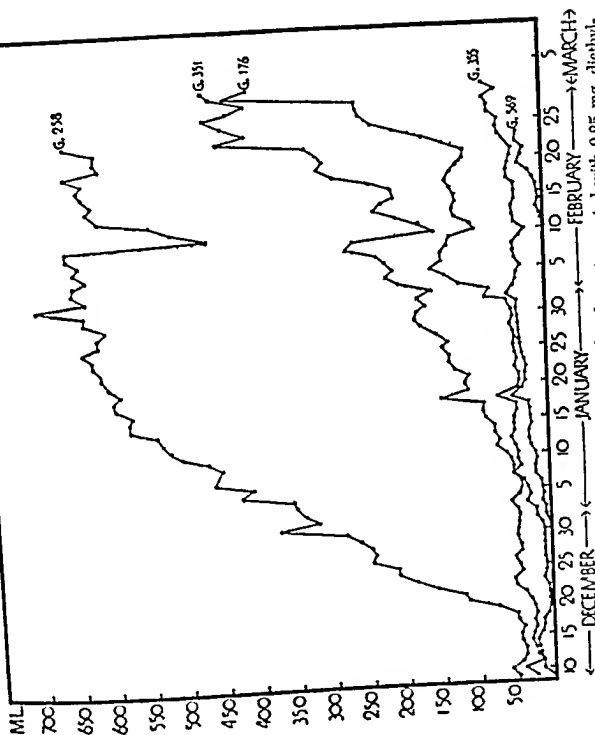


FIG. 19. Milk production curves of 5 virgin female goats injected with 0.25 mg diethylstilbestrol daily. Injections were started on September 8, 1942, and were continued through January 9, 1943. They were reinitiated on February 8, 1943, and were continued through the end of the experiment. Note the wide variation in response (115).

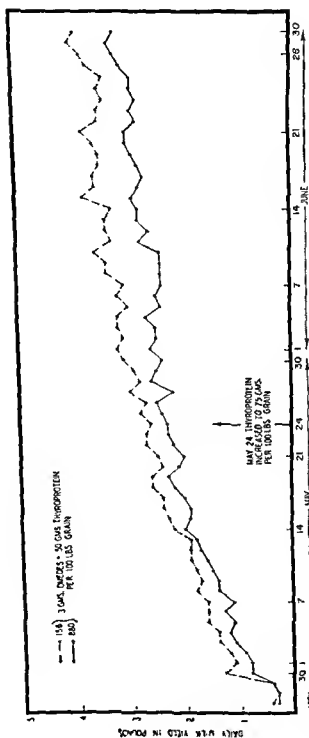


FIG. 20. Experimental induction of lactation in 2 multiparous goats after feeding a dimethyl ether of diethylstilbestrol (DAEDES) and thyroprotein for about 3 months. Hormone feeding was continued during lactation.

3. Effects on Reproductive Function and Health

Administration of estrogens alone to cattle has often induced many of the typical characteristics of nymphomania, including raised tailhead, relaxation of pelvic ligaments and sunken pelvic bones, a tendency to mount or be mounted, and pelvic fractures. Raised tailheads were observed in some cattle by Folley and Malpress (50, 51) and by the Michigan workers (100, 136) even 6 months after termination of estrogen treatment. The ovaries of estrogen-treated cattle have often been found to be small and nonfunctional (68, 93), but cystic follicles and corpora lutea have also been observed in many cases (100).

Meites *et al.* (100) found that implantation of 100 mg. of diethylstilbestrol and 3 gm. of progesterone resulted in some estrous symptoms in about half of 40 treated cattle, while Turner *et al.* (162) reported a complete lack of estrous symptoms with injections of 100 µg. estradiol benzoate and 100 mg. of progesterone (ratio of 1:1000). In agreement with these reports is the observation of Hammond and Day (68) that the symptoms of nymphomania usually were not seen in cattle given estrogens alone if an active corpus luteum was present.

Ovulation, cyst formation, and a gradual return to normal ovarian function have been observed in cattle following cessation of estrogen administration (50, 68, 100). Hammond and Day (68) reported that 34 of 142 cattle which had failed to conceive prior to estrogen implantation became pregnant after about 150 services following removal of the hormones. Folley and Malpress (50) noted that a majority of 32 heifers treated with estrogens became pregnant at the first service after removal of the implants. Cattle and goats treated with both estrogen and progesterone have also conceived following treatment.

No detrimental effects have been observed on the health of animals treated with estrogens and/or progesterone. Body weight gains of from 50 to 300 pounds have been reported in dairy cattle treated with these hormones (136, 163), and weight gains have also been noted in goats given estrogens (86). This might be expected in view of the favorable effects of these hormones on body growth and feed efficiency in beef cattle and sheep (4).

E. Increase of Established Lactation with Hormones

Maximum milk production is usually reached shortly after parturition in most animals, and between one and two months after calving in cattle. Milk yields then decline at a rather constant rate, but with considerable variation among individuals. The causes for the decline in production and the differences in persistency are not entirely clear.

They undoubtedly depend to a large degree on the secretory rate of hormones that influence intensity of lactation, and on factors that maintain the integrity of the mammary secretory tissue.

During the height of lactation, administration of hormones is rarely effective in increasing milk yields, suggesting that hormone secretion rates are not limiting production during this period. Several hormones have been shown to be effective galactopoietic agents during the declining phase of lactation. Turner *et al.* (163) postulated that treatment with a succession of hormones during the declining segment of lactation should make it possible to determine which hormones are limiting the production of individual cows. Presumably, cows that fail to respond to the additional hormones would be secreting adequate amounts of these hormones.

It appears doubtful that the ability of hormones to induce galactopoiesis are necessarily indicative of low endogenous production of these hormones. Most dairy cattle respond to administration of thyroactive substances or STH, with significant increases in milk yield during the declining phase of lactation; yet there is little evidence that these factors are deficient in most dairy cattle. Again, injections of prolactin usually induce only slight galactopoietic effects in dairy cows, while ACTH apparently depresses milk yields; yet both hormones are essential for the maintenance of lactation. The galactopoietic effects of thyroactive substances and STH appear to depend on administering doses in excess of those normally produced by most dairy animals during lactation. There is no evidence that administration of moderate amounts of these hormones are harmful to these animals.

The maintenance or replacement of lobuloalveolar tissue is believed to be an important factor in galactopoiesis. Although hormones that influence alveolar secretory intensity have some ability to maintain the existing mammary parenchyma, there is little evidence that they induce any extensive development of new tissue. This appears to be particularly true of the two most potent galactopoietic substances, STH and thyroactive substances, neither of which has a major role in mammary growth. In the few studies which have been made in laboratory animals, mitotic activity in the mammary gland has been found to be very high during gestation, but virtually disappears after parturition (88, 133).

Can doses of estrogens and/or progesterone be administered to open cows during the declining phase of lactation in amounts sufficient to stimulate new udder growth, without interfering substantially with existing milk production? If this could be done for several months and then terminated, sufficient new mammary tissue might be developed

to evoke a substantial boost in milk yield. The fact that cows can be simultaneously pregnant and lactating, with no noticeable decline in milk production until after the fifth month of gestation, suggests that administration of proper doses of the two hormones may produce a substantial increase in lactation. This combination treatment may be particularly valuable in cows that fail to breed after parturition, but would probably not be suitable for cattle that are both lactating and pregoant. There is some evidence that administration of small amounts of estrogen alone during the declining segment of milk production may increase persistency, and this may be brought about by helping to maintain a high secretion of pituitary prolactin, by inducing growth of new mammary tissue, or by other means.

1. Pituitary Hormones

a. *Whole Anterior Pituitary Extracts.* Whole anterior pituitary preparations elicit increases in milk yield during the declining but not during the peak phase of lactation in dairy cattle and goats. Folley and Young (57) injected the equivalent of 2.5 gm. of fresh anterior pituitary subcutaneously into 85 cows every 2 days for 3 weeks, and noted an increase in milk yield of 20% during the period of injections, and a further 15% increase during the following 2 weeks. A comparison of anterior pituitary extracts from 3 different species indicated that equivalent amounts of horse pituitary were more effective than ox pituitary, while extracts from pig or sheep pituitary depressed milk production in cattle (53). This is of interest, since the anterior pituitary contains at least three hormones, STH, prolactin, and TSH, which can increase milk yields in dairy cattle, while a fourth hormone, ACTH, apparently depresses lactation in this species. It is possible that the variations in pituitary concentration of these four hormones in different species accounted to some degree for the variations noted in response to treatment.

b. *Somatotropin (STH).* When purified STH preparations became available, Cotes *et al.* (26) established that they were highly effective in increasing milk yields in cattle. It was concluded that all or practically all of the galactopoietic activity exhibited by unfractionated ox anterior pituitary extracts in single-injection tests could be attributed to the STH present. There was no indication of a synergistic effect when STH was administered together with prolactin or ACTH (49), although Shaw *et al.* (146) were able to counteract the depressing effects of ACTH on milk yields in cattle with STH.

The British workers suggested that the galactopoietic action of STH

is a reflection of its ability to preserve foodstuffs from oxidation, as in growth and diabetogenesis (20). The galactopoietic action of STH apparently is exerted on the udder itself rather than through any general systemic effects, and it is agreed that STH increases the net efficiency of milk production (22, 25, 75, 146). Turner (personal communication) believes that STH mobilizes all milk precursors for increased milk production. STH does not increase milk yields in lactating rats as measured by litter weight gains, although the body weights of the mother rats are increased (98).

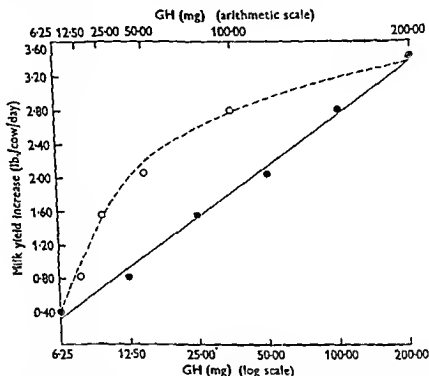


FIG. 21. Relation between log dose of somatotropin (GH) and increase in milk yield in cows (75).

Hutton (75) noted a highly significant linear relationship between the log dose of STH injected and the resultant increase in milk yield in cows (Fig. 21). STH has been reported to increase milk production during the peak as well as during the declining phase of lactation (22), an observation contrary to the results observed with whole anterior pituitary extracts. Shaw (145) reported that injections of STH to heifers for 9 days before and 16 days after parturition increased lactation for the entire remainder of the lactation cycle, but this was not confirmed by Brumby (21).

In ewes, daily intramuscular injections of 25 mg. of STH increased

milk yields from 22 to 40% and significantly increased fat test (82). In lactating goats which had previously been fed thyroprotein for three months, Meites (96) observed that daily injections of 50 mg. of STH increased milk yields by 10 to 15% over the controls, and partially prevented the usual decline in production that follows discontinuation of thyroprotein feeding (Fig. 22).

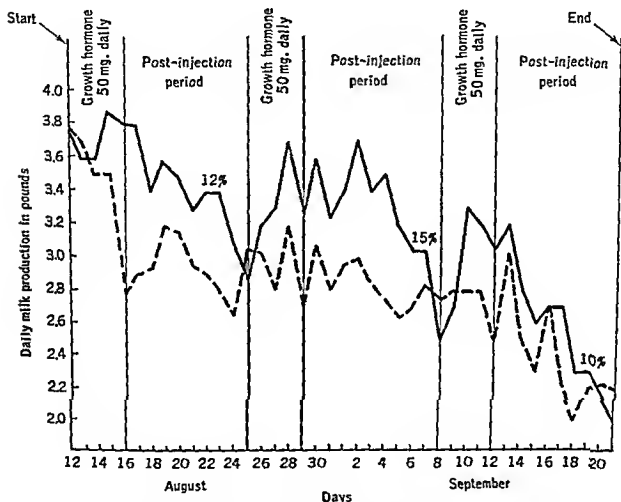


FIG. 22. Partial maintenance of milk production in 4 goats given STH (solid line) as compared to 4 control goats (stippled line). STH injections were begun immediately after stopping thyroprotein, which had been fed for 3 months.

c. *Other Pituitary Hormones.* Prolactin injections have induced only small or moderate increases in milk yield in the cow and goat (5, 56, 150). Prolactin and STH injected together induced a greater increase in milk production in cows than either substance alone (22). TSH caused moderate increase in milk yield in the cow, and injections of TSH and STH together produced greater galactopoietic effects than either substance alone (167).

ACTH injections have usually produced a prompt fall in milk yield in the cow (26, 44, 146). Meites and Reineke (100) noted that injections

for two 7-day periods of 100 mg. of cortisone acetate daily into goats in declining lactation did not alter milk yields. According to Shaw *et al.* (146), the adrenal glucocorticoids may have an important role in regulating milk fat composition in the ruminant, and in the etiology of ketosis in dairy cattle. These workers reported favorable results in treating ketotic cows with these hormones. Further work to determine the effects of ACTH and adrenal cortical hormones on lactation in farm animals would be desirable.

There is some indication that frequent injections of oxytocin may retard the normal decline in milk yields of cows (1, 36, 83). It has long been established that oxytocin injections following normal milking stimulates the release of additional milk with a high fat content. The beneficial effects of oxytocin are usually attributed to a reduction in intra-alveolar pressure, thereby increasing the capacity of the alveolar cells to synthesize milk, but recent work suggests that intra-alveolar pressure does not significantly influence the rate of milk secretion until 16 to 20 hours after milking (40). Since oxytocin does not alter pituitary prolactin secretion (67, 111), it may stimulate other factors favorable to lactation.

2. Thyroactive Substances

Thyroidectomy results in reducing, while administration of thyroactive materials results in increasing, milk yields in cows and goats. The most widely used thyroactive substance is thyroprotein, a biologically active, iodinated casein containing about 1% thyroxine (135). This material has received extensive field trials during the past 15 years and has been approved for commercial use by the Pure Food and Drug Administration. Excellent reviews of its galactopoietic effects have been written by Blaxter *et al.* (14) and Thomas (153).

Synthetic L-thyroxine has recently become available in commercial quantities (24), and British workers (7, 49) believe it has several advantages over thyroprotein. They point out that it is readily obtainable, is active orally, and its purity makes biological assay unnecessary. An oral dose of about 80 mg. of L-thyroxine is stated to have a galactopoietic equivalent of about 20 gm. of thyroprotein in the cow. It remains to be seen whether L-thyroxine will prove to be as economical as thyroprotein or as practical in application.

a. *Factors Influencing Milk Yields.* Many factors influence the ability of thyroactive substances to increase milk yield, including dosage, stage of lactation, length of period of treatment, nutritional status, heredity, age, body weight, temperature, seasonal influences, etc. Blax-

ter (13) concluded that, for doses of 15 to 30 gm. of thyroprotein daily, the response was directly proportional to dose. Doses of 5 gm. daily of thyroprotein were found to produce no significant increase, whereas high doses, up to 80 gm. daily, elicited a large initial rise in milk yield of relatively short duration, accompanied by severe losses in body weight, tachycardia, and elevated body temperatures. The effects of different doses of thyroprotein on milk yield are shown in Fig. 23.

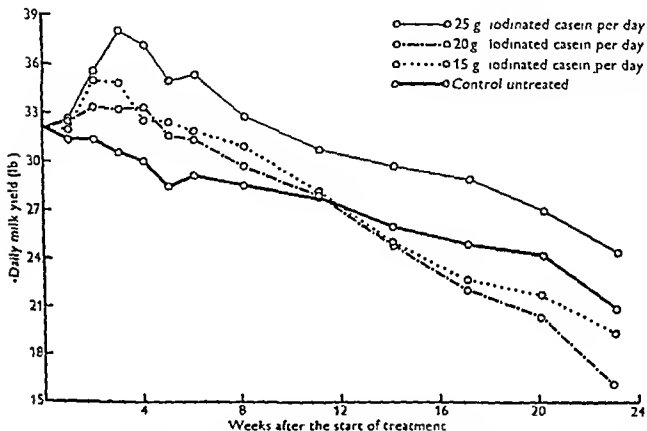


FIG. 23. Effects of different doses of thyroprotein (iodinated casein) on milk yields of groups of 7 cows in declining phase of lactation (81).

When thyroprotein is fed at recommended levels, there is usually a 10 to 25% increase in milk yield, and milk fat is raised by 0.2 to 0.4 percentage points. These increases will usually persist for only two to three months or less if cows are limited in feed intake to their usual levels. If sufficient extra feed is given, a substantial increase in production can be maintained for the entire lactation period. Optimal increases in milk yield are believed to be attained when cows are fed at least 125% of the cow's maximum requirements (153).

According to Blaxter *et al.* (14), smaller animals are stimulated to a greater degree by thyroprotein than larger animals, but older cows show a greater response than younger cows. Animals with good milk producing potential usually respond better than poor producers, and

tained pituitary prolactin levels above that of control parturient rats, although litter weight gain was reduced. Injections of diethylstilbestrol and thyroxine into 2 goats near the end of their lactation periods increased milk yields from less than 2 pounds to between 3 and 4 pounds daily and maintained these levels for a full year (112). Although controls given thyroxine alone were not available, it is doubtful that the thyroxine accounted for all of the observed increase in milk production. Additional evidence has recently been presented that administration of diethylstilbestrol and thyroxine into lactating cows may be more effective in increasing milk production than thyroxine alone (163).

Turner *et al.* (163) fed 5 heifers in declining lactation with 10 mg. of diethylstilbestrol daily for 4 weeks, and reported that the decline continued in some animals for 1 week, followed by a tendency to be arrested and show a slight increase by the fourth week. Browning *et al.* (20) fed 10 mg. of diethylstilbestrol daily to 5 cows, beginning 60 days after parturition and continuing for the following 8 months. Five identical twins were used as controls. The estrogen-treated cows averaged 13% more fat-corrected-milk than the controls, an increase attributed to greater persistency. Wrenn and Sykes (166) similarly fed 10 to 15 mg. of diethylstilbestrol to 12 cows for 60 days during the declining phase of lactation. No changes in daily milk production were noted, but controls were lacking with which to compare persistency.

Small doses of estrogens have been reported to induce an "enrichment" of milk from lactating cows, increasing both total fat and solids-not-fat (45, 147). Apparently there is no change in total milk yield. Hutton (75) recently observed that injections of 12.5 mg., but not of 25.0 mg., of estradiol benzoate daily induced an "enrichment" effect in cows, emphasizing the relatively narrow dosage range of estrogens necessary to produce this effect. The "enrichment" effect may be related to the increases in milk fat observed by Browning *et al.* (20) upon diethylstilbestrol feeding. Turner (personal communication) has recently demonstrated that small amounts of estrogen may increase thyroid function in cows.

There has been considerable speculation as to whether pasture plants contain sufficient amounts of estrogenic substances to influence lactation. Although estrogens have been demonstrated in many plants, there is as yet no convincing evidence that they are present in amounts sufficient to either stimulate or inhibit milk secretion in farm animals. A possible exception may be the subterranean clover found in Australia. This subject has been reviewed recently (16, 128).

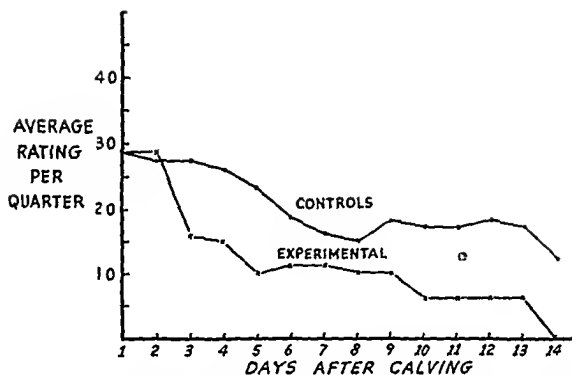


FIG 25 Ratings for udder congestion in 8 heifers treated by incision of the udders with diethylstilbestrol (experimental group) compared to 6 control heifers

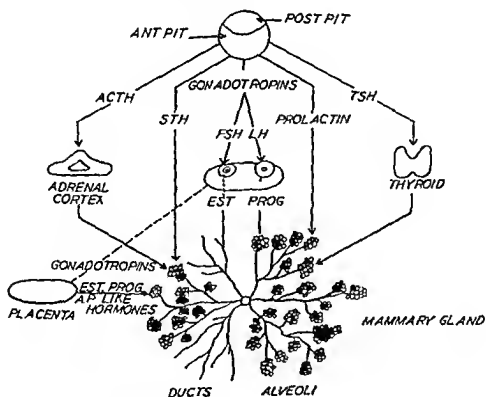


FIG 26 Principal hormones believed to control mammary growth Est = estrogen, Prog = progesterone, AP-like hormones from placenta = AP like hormones other than gonadotropins

with few exceptions, low-producing animals cannot be made into high producers by administering thyroactive substances. Most investigators have found that thyroactive materials are usually less effective in inducing galactopoiesis in hot as compared to cooler climates (14). Smaller amounts may be desirable in warmer climates.

The precise mechanisms by which thyroactive substances exert their galactopoietic effects are unknown. There is little evidence that the gross efficiency of milk production is increased by thyroprotein feeding. The galactopoietic effects of thyroxine have been attributed to an accelerated rate of work by the whole body, increasing feed intake and assimilation, increasing blood circulation and the blood supply to the udder, and stimulating greater activity by the alveolar cells (130). It has been suggested that administration of thyroactive substances may raise prolactin secretion by the pituitary (55), but no effect of thyroxine on the prolactin content of the pituitary was demonstrated in rats (111). The galactopoietic action of thyroactive substances apparently is not mediated through increased STH secretion, since Meites *et al.* (100) observed that administration of optimal doses of thyroprotein to lactating goats was accompanied by an enhanced response if STH was also given. The galactopoietic effects of these two substances appear to be exerted independently of each other (Fig. 24).

b. Effects on General Health and Reproductive Function. Administration of thyroactive substances has usually been found not to be harmful to health, longevity, or reproductive activity. Losses in body weight are related to dosage of thyroactive material given, and cessation of treatment is invariably followed by rapid gains in body weight. Extra feed intake prevents large body weight losses, which can amount to 10 to 15% of original body weight. Increases in heart, pulse, and respiration rates are usual, but electrocardiographic studies have not revealed any damage to the heart (117). No definitely harmful effects on reproductive functions have been established as a result of administering thyroactive substances for short or long periods, or during successive lactations. There are some indications that thyroprotein feeding may actually be beneficial to reproductive functions.

3. Estrogens

Meites and Turner (112) first suggested that administration of small amounts of estrogens during lactation might increase established milk yields by maintaining a high level of pituitary prolactin secretion and inducing new growth of mammary tissue. They reported that injections of diethylstilbestrol for 14 or 21 days into parturient rats main-

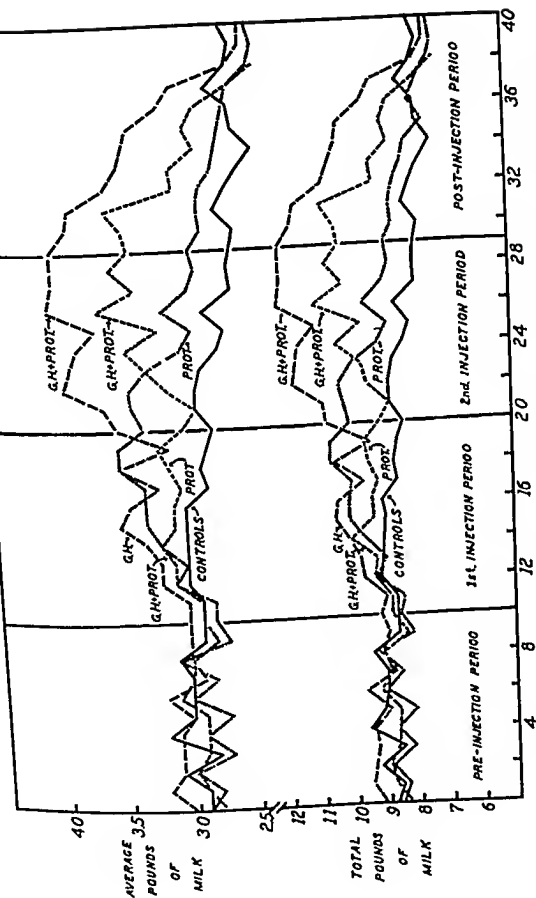


FIG. 24 Effects of thyroprotein (Prot) and/or somatotropin (GH) on lactation in groups of 4 goats each. These results indicate that the effects of thyroprotein and somatotropin are exerted independently of each other. The group (top) was given somatotropin alone during the first injection period, and both hormones during the second period, the group (between) was given both hormones during the first period and only thyroprotein during the second period, the group (bottom) was given only thyroprotein during the first period and received both hormones during the second period.

Inunction of the udder with estrogens may be beneficial in relieving udder congestion frequently encountered after calving. Meites *et al.* (99) massaged the udders of 8 heifers on the first and second days after calving with 200 mg. diethylstilbestrol in 10 ml. corn oil, and compared these with 6 control heifers whose udders were massaged with an equal volume of corn oil. Daily ratings of udder congestion were made, depending on the area of congestion and pliability of the udder. By the

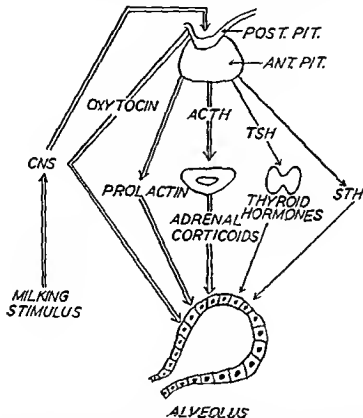


FIG. 27. Nervous and hormonal factors believed to maintain lactation after parturition. The milking stimulus is believed to induce the release primarily of oxytocin, prolactin, ACTH, and adrenal glucocorticoids. Other hormones favorable to lactation may also be released by the milking stimulus. STH and the thyroid exert an important influence on the rate of milk production in some species.

third postpartum day, udder congestion was reduced by half in the estrogen-treated heifers and was completely eliminated by the end of 14 days. By contrast, udder congestion in the control heifers was reduced at a considerably slower rate (Fig. 25). The leucocyte count in the milk of the estrogen-treated heifers was also lowered more rapidly, and there was no effect on milk yield. These effects are believed to be induced by increasing circulation in the udder, thereby relieving venous

and lymphatic stasis. Further work is necessary to determine the effectiveness of this treatment. Diagrammatic representations of the hormonal control of mammary growth and maintenance of lactation are shown in Figs 26 and 27.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr C W Turner, Department of Dairy Husbandry, University of Missouri, and Dr E P Reineke, Department of Physiology and Pharmacology, Michigan State University, for their critical reading of this chapter. Thanks are also due to authors and publishers for permission to use figures.

REFERENCES

- 1 Adams, H P, and Alleo, N N, *J Dairy Sci* 35, 1121 (1952)
- 2 Allan, H, and Wiles, P, *J Physiol (London)* 75, 23 (1932)
- 3 Anderssoo, B, *Acta Physiol Scand* 23, 1 (1951)
- 4 Andrews, F N, Beesoo, W M, and Harper, C, *J Animal Sci* 8, 578 (1949)
- 5 Asdell, S A, Brooks, H J, Salisbury, C W, and Seidenstein, H R, *Cornell Univ Agr Expt Sta Mem No* 198 (1936)
- 6 Averill, S C, Ray, E W, and Lyons, W R, *Proc Soc Exptl Biol Med* 75, 3 (1950)
- 7 Bailey, C L, Bartlett, S, and Folley, S J, *Nature* 163, 800 (1949)
- 8 Baker, B L, and Everett, N B, *Endocrinology* 34, 254 (1944)
- 9 Benson, C K, Cowie, A T, Cox, C P, Flux, D S, and Folley, S J, *J Endocrinol* 13, 46 (1955)
- 10 Benson, C K, Cowie, A T, Cox, C P, and Goldzweig, S A, *J Endocrinol* 15, 126 (1957)
- 11 Benson, C K, and Folley, S J, *J Nature* 177, 700 (1956)
- 12 Benson, C K, and Folley, S J, *J Endocrinol* 14, xi (1957)
- 13 Blaxter, K L, *J Endocrinol* 4, 237 (1945)
- 14 Blaxter, K L, Reineke, E P, Crampton, E W, and Petersen, W E, *J Animal Sci* 8, 307 (1949)
- 15 Boda, J M, and Cole, H H, *J Dairy Sci* 37, 369 (1954)
- 16 Bradbury, R B, and White, D E, *Vitamins and Hormones* 12, 207 (1954)
- 17 Bradley, T R, and Clarke, P M, *J Endocrinol* 14, 28 (1956)
- 18 Braude, R, and Mitchell, K G, *J Endocrinol* 8, 238 (1952)
- 19 Britton, S W, and Kline, R F, *Am J Physiol* 115, 627 (1936)
- 20 Browning, C B, Fountaine, F G, Marion, G B, and Atkeson, F W, *J Dairy Sci* 40, 1590 (1957)
- 21 Brumby, P J, *New Zealand J Sci Technol* A38, 152 (1956)
- 22 Brumby, P J, and Hancock, J, *New Zealand J Sci Technol* A36, 417 (1955)
- 23 Campbell, I L, and Turner, C W, *Missouri Univ Agr Expt Sta Research Bull No* 352 (1942)
- 24 Chalmers, J R, Dickson, G T, Elks, J, and Heims, B A, *J Chem Soc* p 3424 (1949)
- 25 Chung, A C, Shaw, J C, and Gill, W M, *J Dairy Sci* 36, 589 (1953)
- 26 Cotes, P M, Crichton, J A, Folley, S J, and Young, F G, *Nature* 164, 992 (1949)
- 27 Cowie, A T, *J Endocrinol* 16, 135 (1957)
- 28 Cowie, A T, and Folley, S J, *Nature* 156, 719 (1945)
- 29 Cowie, A T, and Folley, S J, *J Endocrinol* 5, 21 (1947)

30. Cowie, A. T., and Folley, S. J., in "The Hormones" (G. Pincus and K. V. Thimann, eds.), Vol. 3, Chapt. 8, p. 309. Academic Press, New York, 1955.
31. Cowie, A. T., and Folley, S. J., in "The Neurohypophysis" (H. Heller, ed.), p. 183. Academic Press, New York, 1957.
32. Cowie, A. T., Folley, S. J., Malpress, F. H., and Richardson, K. C., *J. Endocrinol.* 8, 64 (1952).
33. Cowie, A. T., and Tindal, J. S., *Endocrinology* 56, 612 (1955).
34. Cross, B. A., *J. Endocrinol.* 9, 7 (1953).
35. Cross, B. A., and Harris, G. W., *Nature* 168, 994 (1950).
36. Donker, J. D., Koshi, J. H., and Petersen, W. E., *J. Dairy Sci.* 37, 299 (1954).
37. Dragstedt, L. R., Sudan, A. C., and Phillips, K., *Am. J. Physiol.* 69, 477 (1924).
38. Eaton, O. N., Simmons, V. L., Sykes, J. F., Wrenn, T. R., and Hall, S. R., *J. Dairy Sci.* 36, 1089 (1953).
39. Elias, J. L., *Science* 126, 842 (1957).
40. Elliott, G. M., and Brumby, P. J., *Nature* 176, 350 (1955).
41. Elliott, J. R., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull.* No. 637 (1953).
42. Ely, F., and Petersen, W. E., *J. Dairy Sci.* 24, 211 (1941).
43. Flux, D. S., *J. Endocrinol.* 11, 238 (1954).
44. Flux, D. S., Folley, S. J., and Rowland, S. J., *J. Endocrinol.* 10, 333 (1954).
45. Folley, S. J., *Biochem. J.* 30, 2262 (1936).
46. Folley, S. J., *J. Roy. Soc. Arts* 93, 3 (1944).
47. Folley, S. J., "Marshall's Physiology of Reproduction" (A. S. Parkes, ed.), Chapt. 20, p. 525. Longmans, Green, London, 1952.
48. Folley, S. J., *Recent Progr. in Hormone Research* 7, 107 (1952).
49. Folley, S. J., "The Physiology and Biochemistry of Lactation," C. C Thomas, Springfield, Illinois, 1956.
50. Folley, S. J., and Malpress, F. H., *J. Endocrinol.* 4, 23 (1944).
51. Folley, S. J., and Malpress, F. H., *J. Endocrinol.* 4, 37 (1944).
52. Folley, S. J., and Malpress, F. H., in "The Hormones" (G. Pincus and K. V. Thimann, eds.), Vol. 1, Chapt. 16, p. 745. Academic Press, New York, 1948.
53. Folley, S. J., Malpress, F. H., and Young, F. G., *J. Endocrinol.* 4, 212 (1945).
54. Folley, S. J., Scott-Watson, H. M., and Bottomley, A. C., *J. Physiol. (London)* 98, 15 (1940).
55. Folley, S. J., and White, P., *Proc. Roy. Soc. B120*, 346 (1936).
56. Folley, S. J., and Young, F. G., *Proc. Roy. Soc. B126*, 45 (1938).
57. Folley, S. J., and Young, F. G., *J. Endocrinol.* 2, 194 (1945).
58. Frazier, C. N., and Mu, J. W., *Proc. Soc. Exptl. Biol. Med.* 32, 997 (1935).
59. Gaines, W. L., *Am. J. Physiol.* 33, 285 (1915).
60. Gardner, W. U., and Allen, E., *Anat. Record* 83, 75 (1942).
61. Gardner, W. U., Smith, G. M., and Strong, L. C., *Proc. Soc. Exptl. Biol. Med.* 33, 118 (1935).
62. Gaunt, R., Eversole, W. J., and Kendall, E. C., *Endocrinology* 31, 84 (1942).
63. Gomez, E. T., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* 34, 404 (1936).
64. Coto, T., and Oshima, M., *Bull. Natl. Inst. Agr. Sci. Ser. C, No. 10*, 91 (1955).
65. Cowen, J. W., and Tobey, E. R., *J. Gen. Physiol.* 15, 67 (1931).
66. Gregoire, G., *J. Endocrinol.* 5, 68 (1940).
67. Grosvenor, C. E., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* 97, 463 (1958).

- 68 Hammond, J, and Day, F T, *J Endocrinol* 4, 53 (1944)
- 69 Hancock, J, Brumby, P J, and Turner, C W, *New Zealand J Sci Technol* 36, 111 (1955)
- 70 Hawker, R W, *J Clin Endocrinol and Metabolism* 18, 54 (1958)
- 71 Hibbs, J W, and Pounden, W D, *J Dairy Sci* 34, 498 (1951)
- 72 Hohn, E O, *J Endocrinol* 16, 227 (1957)
- 73 Hooker, C W, and Williams, W L, *Endocrinology* 28, 42 (1941)
- 74 Houssay, B A, *Compt rend soc biol* 120, 496 (1935)
- 75 Hutton, J B, *J Endocrinol* 17, 121 (1958)
- 76 Johnson, R M, Ph D thesis, Michigan State Univ, East Lansing, Michigan, 1957
- 77 Johnson, R M, and Meites J, *Proc Soc Exptl Biol Med* 89, 455 (1955)
- 78 Johnson, R M, and Meites, J, *J Animal Sci* 16, 1288 (1956)
- 79 Johnson, R M, and Meites, J, *J Animal Sci* 16, 72 (1957)
- 80 Johnson, R M, and Meites, J, *J Dairy Sci* 40, 625 (1957)
- 81 Johnston, R F, and Smithcors, J F, *Endocrinology* 43, 193 (1948)
- 82 Jordan, R M, and Shaffhausen, D D, *J Animal Sci* 13 706 (1954)
- 83 Knodt, C B, and Petersen W E, *J Dairy Sci* 27, 449 (1944)
- 84 Leech, F B, *J Endocrinol* 7, 42 (1950)
- 85 Lewis, A A, and Turner, C W, *J Dairy Sci* 24, 845 (1941)
- 86 Lewis, A A, and Turner, C W, *J Dairy Sci* 25, 985 (1942)
- 87 Liu, T Y, and Turner, C W, *J Dairy Sci* 32, 881 (1949)
- 88 Loeb, L, and Hesselberg, C, *J Exptl Med* 25 285 (1917)
- 89 Lyons, W R, *Proc Soc Exptl Biol Med* 51, 308 (1942)
- 90 Lyons, W R, Chaikoff, I L, and Reichert, F L, *Proc Soc Exptl Biol Med* 31, 303 (1933)
- 91 Lyons, W R, Johnson R E, Cole, R D, and Li, C H, in "The Hypophyseal Growth Hormone, Nature and Actions" (R W Smith, O H Gaebler, and C N H Long, eds), p 461 McGraw Hill, New York, 1955
- 92 Lyons, W R, Li C H, and Johnson R E, *J Clin Endocrinol and Metabolism* 13, 836 (1953)
- 93 Marshall, S P, Becker, R B, Dix Aronald, P T, and Sanders, D A, *Florida Univ Agr Exptl Stas (Gainesville) Bull No* 440 (1948)
- 94 McQueen-Williams, M, *Proc Soc Exptl Biol Med* 33, 406 (1935)
- 95 Meites, J, *Rev can biol* 13, 359 (1954)
- 96 Meites, J, in "The Hypophyseal Growth Hormone, Nature and Actions" (R W Smith, O H Gaebler, and C N H Long, eds), p 493 McGraw Hill, New York, 1955
- 97 Meites, J, *Ann endocrinol (Paris)* 17, 519 (1956)
- 98 Meites, J, *Proc Soc Exptl Biol Med* 96, 730 (1957)
- 99 Meites, J, Horwood R E, Reineke E P, Bryan, C S, and Smiley, E S, *J Dairy Sci* 33, 383 (1950)
- 100 Meites, J, Reineke, E P, and Curry, C F, unpublished observations, 1950
- 101 Meites, J, Reineke, E P, and Huffman, C F, *Mich State Univ Agr Exptl Sta Quart Bull* 32 115 (1950)
- 102 Meites, J, and Sgouris J T, *Endocrinology* 63 17 (1953)
- 103 Meites, J, and Sgouris J T, *Endocrinology* 65 530 (1954)
- 104 Meites, J, Trentin, J J, and Turner, C W, *Endocrinology* 31 607 (1942)
- 105 Meites, J, and Turner C W, *Endocrinology* 30 711 (1942)

106. Meites, J., and Turner, C. W., *Endocrinology* 30, 728 (1942b).
107. Meites, J., and Turner, C. W., *Endocrinology* 30, 719 (1942c).
108. Meites, J., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* 49, 193 (1942).
109. Meites, J., and Turner, C. W., *Am. J. Physiol.* 150, 394 (1947).
110. Meites, J., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* 64, 488 (1947).
111. Meites, J., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 415* (1948).
112. Meites, J., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 416* (1948).
113. Meites, J., and Turner, C. W., in "Hormone Assay" (C. W. Emmens, ed.), p. 237. Academic Press, New York, 1950.
114. Miller, W. R., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* 90, 142 (1955).
115. Mixner, J. P., Meites, J., and Turner, C. W., *J. Dairy Sci.* 27, 957 (1944).
116. Mixner, J. P., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 378* (1943).
117. Mullick, D. N. B., Alfredson, B. V., and Reineke, E. P., *Am. J. Physiol.* 152, 100 (1948).
118. Munson, P. L., *Ann. N. Y. Acad. Sci.* 60, 776 (1955).
119. Nellor, J. E., and Reineke, E. P., *J. Dairy Sci.* 41, 789 (1958).
120. Nelson, W. O., *Physiol. Revs.* 16, 468 (1938).
121. Nelson, W. O., *Colloq. intern. centre, natl. recherche sci. (Paris)* No. 32, 19 (1950).
122. Nelson, W. O., and Caunt, R., *Proc. Soc. Exptl. Biol. Med.* 34, 871 (1938).
123. Nelson, W. O., Himwich, H. E., and Fazekas, J. F., *Anat. Record* 66, 201 (1936).
124. Newton, W. H., and Richardson, K. C., *J. Endocrinol.* 2, 322 (1941).
125. Petersen, W. E., *J. Dairy Sci.* 25, 71 (1942).
126. Petersen, W. E., Knodt, C. B., Ludwick, T. M., and Pomeroy, B. S., *Proc. Soc. Exptl. Biol. Med.* 57, 332 (1944).
127. Petersen, W. E., and Ludwick, T. M., *Federation Proc.* 1, 68 (1942).
128. Pope, C. S., *Dairy Sci. Abstr.* 16, 334 (1954).
129. Poulton, B. R., and Reece, R. P., *Endocrinology* 61, 217 (1957).
130. Ralston, N. P., Cowsert, W. C., Ragsdale, A. C., Herman, H. A., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 317* (1940).
131. Reece, R. P., and Turner, C. W., *Proc. Soc. Exptl. Biol. Med.* 34, 402 (1936).
132. Reece, R. P., and Turner, C. W., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 266* (1937).
133. Reece, R. P., *J. Dairy Sci.* 39, 726 (1956).
134. Reece, R. P., Bartlett, J. W., Hathaway, I. L., and Davis, H. P., *Proc. Soc. Exptl. Biol. Med.* 43, 183 (1940).
135. Reineke, E. P., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 355* (1942).
136. Reineke, E. P., Meites, J., Cairy, C. F., and Huffman, C. F., *Proc. Book Am. Vet. Med. Assoc.* 89, 325 (1952).
137. Richardson, K. C., *Proc. Roy. Soc. B* 136, 30 (1949).
138. Richardson, K. C., *J. Endocrinol.* 9, 170 (1953).
139. Riddle, O., Bates, R. W., and Dykshorn, S. W., *Anat. Record* 54, 25 (1932).
140. Scharf, C., and Lyons, W. R., *Proc. Soc. Exptl. Biol. Med.* 48, 86 (1941).
141. Selye, H., *Am. J. Physiol.* 107, 535 (1934).
142. Selye, H., *Acta Endocrinol.* 17, 394 (1954).
143. Sgouris, J. T., and Meites, J., *Am. J. Physiol.* 175, 319 (1953).

- 144 Sgours, J T, and Meites, J, *Am J Physiol* 169, 301 (1952)
- 145 Shaw, J C, in "The Hypophyseal Growth Hormone, Nature and Actions" (R W Smith, O H Gaebler, and C N H Long, eds), p 486 McGraw-Hill, New York, 1955
- 146 Shaw, J C, Chung, A C, and Bunding, I, *Endocrinology* 3, 327 (1955)
- 147 Spielman, A, Ludwick, T M, and Petersen, W E, *J Dairy Sci* 24, 499 (1941)
- 148 Stricker, P, and Grueter, F, *Compt rend soc biol* 99, 1978 (1928)
- 149 Sykes, J F, in *Hormonal Relationships and Applications in the Production of Meats, Milk and Eggs* Agr Board, Natl Research Council, Publ 266, p 41, Washington, D C, 1953
- 150 Sykes, J F, Meuleman, W L, and Huffman, C F, *Endocrinology* 30, 217 (1942)
- 151 Sykes, J F, and Wrenn, T R, *J Dairy Sci* 34, 1174 (1951)
- 152 Tabachnik, I A, and Trenton, J J, *Federation Proc* 10, 339 (1951)
- 153 Thomas, J W, in "Hormonal Relationships and Applications to the Production of Meats, Milk and Eggs," Agr Board, Natl Research Council Publ 266, p 47, Washington, D C, 1953
- 154 Trenton, J J, and Turner, C W, *Missouri Univ Agr Expt Sta Research Bull No 418* (1948)
- 155 Turner, C W, in "Hormone Assay" (C W Emmens, ed), p 261 Academic Press, New York, 1950
- 156 Turner, C W, in "Sex and Internal Secretions" (E Allen, ed), 2nd ed, p 740 Williams & Wilkins, Baltimore, Maryland, 1939
- 157 Turner, C W, and Gardner, W U, *Missouri Univ Agr Expt Sta Research Bull No 168* (1931)
- 158 Turner, C W, and Meites, J, *Endocrinology* 29, 165 (1941)
- 159 Turner, C W, and Meites, J, *Proc Soc Exptl Biol Med* 47, 232 (1941)
- 160 Turner, C W, and Reincke, E P, *Missouri Univ Agr Exptl Sta Research Bull No 225* (1936)
- 161 Turner, C W, and Slaughter, I S, *J Dairy Sci* 13, 8 (1930)
- 162 Turner, C W, Yamamoto, H, and Ruppert, H L, *J Dairy Sci* 39, 1717 (1956)
- 163 Turner, C W, Yamamoto, H, and Ruppert, H L, *J Dairy Sci* 40, 37 (1957)
- 164 Walker, S M, and Stanley, A J, *Anat Record* 78, 142 (1910)
- 165 Whittlestone, W G, Bassett, E G, and Turner, C W, *J Dairy Sci* 35, 889 (1952)
- 166 Wrenn, T R, and Sykes, J F, *J Dairy Sci* 40, 1581 (1957)
- 167 Wrenn, T R, and Sykes, J F, *J Dairy Sci* 36, 1313 (1953)
- 168 Zeckwer, I T, *Science* 100, 123 (1911)
- 169 Zondk, B, *Nature* 164, 154 (1914)

Author Index

Numbers in parentheses are reference numbers and are included to assist in locating the reference where the authors' names are not mentioned in the text. Numbers in italics refer to the page on which the reference is listed.

A

- Abraham, A. R., 125 (218), 126 (218), 153
- Abrahams, V. C., 216 (1), 216
- Abramovich, C. E., 454, 466
- Abrams, M. E., 190, 193, 216
- Abramson, D., 147 (1), 147, 532 (1), 533
- Abreu, B., 526 (163), 531 (164), 537
- Acher, R., 530 (2), 533
- Ackermann, E., 29 (94), 51 (94), 57
- Adams, C. E., 473 (117), 504
- Adams, C. W. M., 257, 260
- Adams, E., 346, 355
- Adams, H. P., 582 (1), 589
- Addison, W. H. F., 122 (2), 147
- Aehneit, E., 270 (1), 277 (1), 287
- Ahlquist, R. P., 531 (164), 537
- Aitken, Elsie H., 117 (203), 152
- Aitken, W. A., 268 (2), 278 (2), 287
- Ajello, P., 329 (1), 329
- Albert, A., 90 (7, 8, 9), 95 (2), 96 (2), 100 (1), 102 (7, 8, 9), 103 (7, 8, 9), 106, 146 (3), 147, 173 (102), 174 (102), 182
- Alexander, F., 247, 260
- Alexander, G., 515, 533
- Alexander, G. L., 196 (25), 217
- Alexander, M. H., 512 (4), 517 (4), 533
- Alfredson, B. V., 584 (117), 592
- Allan, H., 547 (2), 589
- Allden, W. G., 292, 329
- Allen, A. D., 341, 353, 355
- Allen, B. M., 34 (1), 55
- Allen, E., 131, 132, 135, 147, 148, 297 (80), 331, 463 (58), 464 (58), 468, 472 (1), 477 (69), 501, 503, 547 (60), 590
- Allen, N. N., 582 (1), 589
- Allen, W. M., 529 (67), 530 (5), 533, 535
- Altman, M., 349, 355
- Altschule, M. D., 212, 219
- Amoroso, E. C., 360 (1), 364 (45), 374, 379 (45), 380 (45), 392, 393, 395, 423, 424, 426, 430, 431, 437 (2, 38), 448, 450 (2, 38), 451, 453, 457 (2), 458 (2), 459 (2), 460, 461 (2), 466, 467, 497 (2), 499 (2), 501
- Amsbaugh, A. E., 349 (19), 355
- Ance, P., 495, 502
- Andersen, A. C., 359 (7), 364 (2), 365 (4), 367 (5, 6), 376 (2), 385 (3, 8), 393 (2), 393, 394
- Andersen, D., 360 (9), 374 (9), 394
- Anderson, E., 196 (137), 220
- Anderson, E. M., 77 (36), 107
- Anderson, J., 297 (3), 329
- Anderson, L. L., 353 (22), 356
- Anderson, O. D., 366, 382 (10), 394
- Andersson, B., 216 (3), 216, 559, 589
- Andres, J., 520, 533
- Andrews, F. N., 40 (79), 57, 162, 171, 180, 182, 271, 272, 273, 277, 279 (3), 280 (3), 282, 283, 284, 285, 286 (3), 287, 408 (62), 432, 525 (7), 534, 577 (4), 589
- Anthoff, H. R., 206, 216
- Antopol, W., 173 (2), 180
- Appleby, A., 310 (40), 313 (40), 330
- Arenas, N., 143 (6), 148, 364 (11), 370 (11), 371, 372, 373 (11), 374 (11), 376, 378 (11), 382, 383, 386, 387, 388, 389 (11), 391 (11), 394
- Arey, L. B., 41 (2), 55
- Armstrong, D. T., 224 (167), 225 (89, 167), 226 (89), 230 (167), 231 (167), 234 (167), 236 (167), 247 (89), 249 (89), 253 (3), 258, 260, 262, 264
- Arnold, J. P., 41 (3), 55
- Aron, C., 20 (1), 23
- Aron, M., 20 (1), 23, 166, 170, 180
- Aschbacher, P. W., 226, 227, 260

- Aschheim, S., 66, 94 (3), 106, 110, 487
(3, 4), 501
- Aschner, B., 191, 216, 386 (12), 394,
477 (5), 501
- Asdell, S. A., 2 (2), 23, 119 (7), 129
(7), 139 (7), 141, 143, 144 (48),
145 (7), 148, 149, 225, 226, 228,
229 (8), 231 (83), 234 (7), 230
(7), 238, 239 (6, 8), 241 (82), 244,
245, 246, 247, 253, 254 (6), 260,
261, 282, 264, 267, 268 (4, 5, 6),
271, 277 (4), 278 (4), 280 (4), 282,
283, 286 (4, 6), 287 (4), 287, 294
(4), 295, 297, 300 (4), 303 (59),
328 (4, 5), 329 (5), 329, 331, 384
(13), 389 (14), 390 (14), 393
(13), 394, 472 (6), 501, 513 (8),
515 (9), 517 (9), 518 (19), 519
(8), 534, 581 (5), 589
- Asmundson, V. F., 169, 180
- Assenmacher, I., 189 (23, 24), 216, 217,
257, 260
- Assheton, R., 299 (6), 329 (6), 329,
447 (3), 455, 458, 466
- Astwood, E. B., 74 (4), 75 (5), 106,
131 (8), 136, 143, 148, 472 (7, 9),
477 (8), 502
- Atalla, F., 161, 180
- Atkeson, F. W., 586 (20), 589
- Atkinson, W. B., 472 (10, 19), 502
- Austin, C. R., 82 (6), 106, 227, 260, 400
(3, 4, 16, 61), 403, 404, 408 (3),
409 (3, 5, 7), 412 (5), 413 (8, 16),
415, 418 (6), 431, 432
- Austin, P. R., 88 (53), 90 (54), 101
(54), 107
- Autrup, E., 228 (10), 260
- Avenill, R. L. W., 297 (7), 329, 429 (9),
431
- Averill, S. G., 83 (131), 109, 546, 589
- Aylward, F., 489, 502
- B**
- Bachman, C., 117 (9), 148
- Baclesse, M., 174 (27), 180
- Bahn, R. C., 90 (7, 8, 9), 106
- Bahr, C. F., 23 (4), 23
- Bailey, C. L., 163, 181, 582 (7), 589
- Bailey, P., 192, 216
- Bailey, W. W., 176 (93), 177 (92, 93),
162
- Bajez, E., 354 (4), 355
- Baker, B., Jr., 336 (30), 356
- Baker, B. L., 550 (8), 569
- Baker, J. R., 187, 216
- Baker, L. N., 340, 341, 354 (5), 355
- Balard, P., 527 (10), 534
- Balfour-Lynn, S., 427, 431
- Balfour, W. T., 470 (12), 487 (12), 502
- Ball, J., 178, 181, 207 (8), 216
- Baltus, B. J., 76 (153, 154), 110
- Barclay, A. E., 36 (37), 56
- Bard, P., 191 (9), 204 (9), 207 (9),
208, 216, 527 (11), 534
- Bargmann, W., 257, 260
- Barker, W. L., 343 (7), 344, 355
- Barkes, A. S., 198 (75), 199 (75, 76),
218
- Barlow, G., Jr., 193 (190), 213 (191),
221
- Barnes, C. M., 496 (13, 75a), 502, 503
- Barracrough, C. A., 201 (10, 11, 12), 216
- Barrett, G. R., 226 (119), 257, 283
- Bartelmez, G. W., 19 (24), 22 (5, 6),
23, 24
- Bartlett, J. W., 544 (134), 592
- Bartlett, S., 582 (7), 589
- Barton, E. P., 369 (15), 373, 394
- Bascich, P., 6 (3), 8 (3), 23
- Bascom, K. F., 33 (4), 55
- Bassett, E. G., 307, 329, 533 (12), 534,
558 (165), 559 (165), 593
- Bates, R. W., 547 (139), 592
- Batson, O. V., 42 (5, 6), 55
- Bauer, H. C., 191, 212, 216
- Baum, H., 2 (34), 24, 457, 467
- Bayley, N. D., 228 (112), 263
- Beach, F. A., 115 (10), 148, 205 (15,
16), 206 (15, 16, 17), 207, 210, 211
(18, 21, 22), 216, 365, 394
- Beach, V. L., 532 (147), 537
- Beadenkopf, W. G., 200 (38), 217
- Bearden, H. J., 225 (85, 103), 226
(103), 227, 230 (12), 255 (85,
103), 260, 262
- Bearse, C. E., 169, 172, 180
- Beattie, J., 209 (45), 217
- Beatty, R. A., 417 (11), 427 (11), 431

- Beck, A B, 501 (14), 502
 Becker, R B, 565 (93), 568 (93), 569 (93), 591
 Beer, G, 456 (4), 463 (4), 466
 Beeson, W M, 577 (4), 589
 Bell, G H, 531 (13), 534
 Bell, T D, 310 (10), 323 (11), 329, 330, 473 (15), 502
 Bell, W B, 386 (17, 18), 394
 Bellotti, R, 116 (207), 153
 Benesch, F, 354 (8), 355
 Bennet, H S, 446, 468
 Bennett, W A, 90 (7, 8, 9), 102 (7, 8, 9), 103 (7, 8, 9), 106
 Bennetts, H W, 132 (11), 148
 Benoit, J, 166, 170, 180, 189, 216, 217, 257, 260
 Benson, G K, 258, 260, 491, 502, 540 (9), 541 (9, 10), 542, 543 (9), 553 (11, 12), 568, 589
 Benzie, D, 462 (5), 463 (5), 466
 Berg, L R, 169, 172, 180
 Berg, O A, 47, 55
 Bergensdal, D M, 78 (10), 106
 Bergmann, R, 465, 466
 Berkson, J, 95 (2), 96 (2), 106
 Berliner, V R, 164, 180, 268 (7), 269 (7, 9), 270 (8, 14), 271 (13), 273 (7, 9), 275 (7, 8, 9), 277 (7, 12, 14), 286 (7, 9, 10, 11, 12, 13), 287, 408 (62), 432
 Bern, H A, 30, 55
 Bernstein, J, 8 (100), 12, 26
 Berry, C M, 197 (62), 198 (62), 218
 Berry, B O, 22 (88a), 25, 230 (128), 263, 343 (52), 356, 437 (63), 468, 490 (130), 505
 Berthelon, M, 388 (66), 395
 Berthrong M E, 34 (10), 55
 Berwind, T, 22 (7), 23
 Bhattacharyya, P, 252 (80), 253 (80), 262
 Bilet-Laprida, Z, 134 (12), 148
 Buck, J, 169 (15), 180
 Biggart, J H, 190 (25), 217
 Biggers, J D, 117 (112), 131 (13, 14, 112), 148, 150
 Bilderback, J B, 212 (105), 220
 Biller, H V, 158 (12, 13, 14), 170 (14), 171, 172, 160
 Binet, F E, 309 (126), 315 (126), 316 (126), 317 (126), 318 (126), 332
 Bird, H R, 169 (67), 181
 Bischoff, T L W, 373, 394, 450, 466
 Bishop, D W, 410 (15), 430 (12, 13, 14), 431
 Bishop, K S, 87 (48), 107
 Bishop, M W H, 400 (3, 4, 16), 403, 404, 408 (3), 409 (3, 5), 412 (5), 415, 431
 Biskind, M S, 87 (11), 106
 Bissonnette, T H, 187, 188 (28), 189 (29), 190 (27), 217, 328 (12), 330
 Bitman, J, 254, 260
 Black, W G, 246 (17, 19, 20, 21), 252, 260
 Black, W H, 514 (92), 517 (92), 536
 Blair, A, 115 (15), 148
 Blair, G W S, 242, 260
 Blakeslee, L H, 270 (15), 280 (15), 287
 Blaudau, R J, 82 (12), 106, 178, 180, 299 (97), 332
 Blau, N F, 379, 396
 Blaxter, K L, 478 (17), 502, 582, 583, 584 (14), 589
 Bligh, E G, 114 (120), 150
 Blivaiss, B B, 170, 172, 180
 Bloch, K, 115 (16), 148
 Bloch, S, 473 (18), 502
 Block, E, 5 (8), 23
 Bloom, F, 389 (20), 390 (20, 21), 391 (21), 392 (21), 394
 Bloor, W B, 343, 355
 Blotner, H, 215 (30), 217
 Blum, C T, 513, 517 (23), 534
 Blyth, J S S, 128 (100), 150
 Bo, W J, 472 (19), 502
 Bocciabello, B A, 1 (67), 25
 Boda J M, 22 (23), 24, 70 (130), 88 (26, 30), 88 (130), 106, 107, 109, 155 (131), 467, 199 (32a), 502, 555 (15), 559
 Bodansky, M, 478 (20), 502
 Boeltinger, E G, 131 (17), 148
 Boving, B G, 470 (21), 502
 Bogart, B, 104 (20), 160, 229 (113), 263
 Bogdanova M P, 352 (31), 356

- Bogdanove, E. M., 102 (31, 32), 196
 (31, 32), 197 (31), 213 (32a), 217
 Bohstedt, G., 310 (10), 329
 Bojar, S., 200 (38), 217
 Bollman, J. L., 47 (84, 85), 57
 Bonadonna, T., 512 (14), 517 (14), 534
 Bone, J. F., 242, 260
 Bonfert, A., 225 (26, 27), 228 (27),
 260, 261
 Bongiovanni, A. M., 116 (41), 148
 Bonham, C., 529 (52), 535
 Bonnet, R., 450, 455, 459, 466
 Bonsma, F. N., 517 (15), 518 (15), 534
 Bonsma, J. C., 513, 517 (85), 518 (85),
 535
 Bonsnes, R. W., 76 (168), 110
 Booker, E. E., 168, 180
 Boot, L. M., 193 (194), 221
 Borell, U., 21 (9, 10), 23
 Bottomley, A. C., 125 (18), 148, 566
 (54), 590
 Boulin, P., 495, 502
 Boulware, R., 251 (178), 265
 Bourdelle, E., 29 (11, 73, 74), 55, 57
 Bourgarel, R., 529 (16), 534
 Bourne, G., 249, 261
 Bove, E. R., 20 (33), 24
 Bowden, F. P., 187, 220
 Boyd, E. M., 344, 355
 Boyd, J. D., 373 (22), 394, 400 (17),
 422, 424, 431
 Boyd, W. L., 165 (80), 181
 Boyden, E. A., 11 (11), 23
 Boyland, E., 243 (29), 261
 Bradbury, J. T., 75 (15), 106, 496 (96),
 504
 Bradbury, R. B., 586 (16), 589
 Braden, A. W. H., 178, 180, 227, 260,
 409 (7), 413 (8, 18), 418 (6), 431
 Bradley, O. C., 370 (23), 371 (23), 383
 (23), 394
 Bradley, T. R., 548, 589
 Bradshaw, T. E. T., 126 (70), 134, 149
 Brady, R. O., 114 (19), 148
 Brakel, W. J., 511 (17), 512, 514 (17),
 516 (17), 517 (17), 518, 534
 Brambell, F. W. R., 2 (12), 23, 139
 (46), 142, 143, 148, 231, 233 (30),
 261, 373 (24), 382 (24), 394, 405
 (19), 429 (20), 431, 471 (23), 502
 Brandt, G., 512 (123), 536
 Branton, C., 226, 261
 Bratt, H. M., Jr., 390, 394
 Bratton, R. W., 224 (167), 225 (167),
 230 (12, 167), 231 (167), 236
 (167), 260, 264
 Braude, R., 516 (18), 534, 558 (18),
 589
 Braunsberg, H., 137 (22), 148
 Bremer, F., 102, 216
 Bremer, J. L., 444 (10), 445, 468
 Breneman, W. R., 79 (13), 106, 121
 (23), 148
 Brennan, D. M., 355 (69), 357, 532
 (169), 538
 Bretschneider, W., 338, 339, 340, 347
 (47), 349 (47), 356
 Brewer, D. B., 531 (19), 534
 Briggs, H. M., 204 (13), 297 (13), 330,
 497 (108), 504
 Brill, L., 130 (24), 148
 Brinkman, D. C., 253 (74), 282
 Britton, J. W., 275, 276 (17), 277 (16),
 287, 518, 520 (20), 534
 Britton, S. W., 554 (19), 589
 Brobeck, J. B., 199 (105), 221
 Brobeck, J. R., 196 (140), 220
 Brock, H., 251 (32), 252 (32), 253, 261
 Brody, S., 183 (24), 169 (23), 180,
 250 (33), 261, 464, 466
 Bromberg, Y. M., 94 (14), 96 (14), 108
 Brookhart, J. M., 197 (35), 208, 210,
 217
 Brooks, C. M., 193 (37), 199, 200, 207
 (36), 208 (36), 217
 Brooks, H. J., 581 (5), 589
 Brouha, L., 147 (25), 148, 386 (26),
 394
 Brown, C. H., 212 (193), 221
 Brown, J. B., 90 (104), 100 (104), 109
 Brown, M. M., 165 (137), 183
 Brown, W. E., 75 (15), 106
 Brown, W. H., 527 (21), 534
 Browne, J. S. L., 75 (16), 106, 137
 (224), 153, 487, 492 (42), 502
 Browning, C. B., 586, 589
 Brumby, P. J., 559 (40), 568 (69), 569
 (69), 580, 581 (22), 582 (40),
 589, 590, 591
 Bruner, J. A., 89 (17), 103 (17), 106

- Bryan, C S, 581 (99), 588 (99), 591
 Brzezinski, A, 94 (14), 96 (14), 106
 Buch, N C, 228 (34), 229, 261, 526
 (22), 534
 Buckner, P J, 252 (191), 253 (192),
 265
 Bucy, P C, 210, 219
 Bukovics, E, 94 (18), 96 (18), 106
 Bullard, J F, 355 (69), 357, 532 (169),
 538
 Bulbring, E, 136, 148
 Bulgrin, K D, 225 (27), 228 (27), 261
 Bullough, W S, 117 (27, 28), 148
 Bulmer, D, 13 (13, 14), 23
 Bunding, I, 579 (146), 580 (146), 581
 (146), 582 (146), 593
 Bunn, J P, 192, 217
 Burburk, R C, 123 (152), 151
 Burch, C R, 390, 394
 Burger, J F, 178 (25), 180, 337, 338,
 339, 340, 341, 342, 349, 350, 355
 Burgos, M H, 22 (151), 27, 34 (29), 56
 Burkhhardt, J, 269 (18, 19), 270, 271,
 278 (18, 19, 20), 279, 281, 286 (19,
 20), 287
 Burkl, W, 19, 23
 Burmester, B R, 172 (105), 182
 Burn, J H, 136, 148
 Burns, R K, 13 (18), 15 (19, 20, 201),
 23, 24, 495 (25), 502
 Burn, K, 270 (21), 277 (21), 287
 Burrell, M W, 127 (97), 128, 137 (96,
 98, 99), 150
 Burnis, M J, 513, 517 (23), 534
 Burrows, H, 117 (29), 122, 131, 133
 (29, 30), 148
 Burrows, W H, 160, 182
 Bush, L F, 294 (32), 336
 Bustad, L K, 496 (13, 751), 562, 563
 Butenandt, A, 126 (31), 148
 Butler, O D, 22 (881), 25, 230 (128),
 263, 437 (63), 468, 490 (130), 505
 Butt, W R, 140 (32), 148
 Byrnes, W W, 79 (10, 20), 106, 207
 (40), 217
- C
- Cairev, C F, 139 (209), 153, 568
 (100), 571 (100, 130), 577 (100,
 136), 581 (100), 591, 592
 Cajal, S R, 193, 217
 Caligaris, L C S, 487 (190), 506
 Callow, N H, 129 (33), 148
 Callow, R K, 128 (100), 129 (33), 148,
 150
 Cameron, H S, 286 (22), 287
 Campbell, D M, 393 (27), 394
 Campbell, I L, 555, 589
 Camus, J, 191, 192 (42), 217
 Camus, L, 124, 148
 Candinas, L, 354 (53, 54), 356
 Cann, M C, 114 (120), 150
 Cantarow, A, 482 (149), 484 (149),
 505
 Carlo, J, 492 (78), 494 (78), 503
 Carlson, A J, 387 (58), 389 (58, 59),
 390 (59), 395
 Carmon, J L, 34 (12), 55
 Carr, E B, 13 (21), 24
 Carstens, H B, 258 (107), 263
 Carter, F, 83 (21), 103 (143), 104
 (143), 106, 110, 476 (171), 506
 Cartland, G F, 90 (22), 106
 Carvaghios, R, 232 (35), 261
 Casida, L E, 144 (36), 148, 173, 180,
 224 (95), 228 (34, 47), 229, 230
 (36), 246 (17, 10, 20), 251 (40,
 177), 252 (18, 38, 39, 40), 253
 (166), 255 (160), 257, 260, 261,
 262, 264, 265, 297 (80), 298 (14,
 39), 299 (14), 310 (10, 03, 150),
 311 (33, 98), 323 (11), 326 (15,
 33, 93, 98), 327 (33), 328 (98),
 329, 336, 331, 332, 333, 336, 337
 (49, 62), 338 (45, 49, 62, 66), 339
 (45), 340 (6, 61), 341 (6), 350
 (45, 49, 62, 71), 351 (40, 71), 352
 (58), 353 (60), 354 (5, 34), 355,
 356, 357, 473 (15), 475 (137), 476
 (137), 501 (66), 502, 503, 505,
 525 (24), 526 (22), 531
 Cackel, E, 268 (23), 269, 287
 Cathepole, H R, 34 (15), 55, 115 (37),
 122 (37), 148, 472 (29, 135), 473
 (71), 474 (152), 475 (26), 476
 (31), 488 (28), 429 (39), 493
 (31), 494 (29, 71), 495 (29, 39),
 498 (31, 39), 499 (27), 502, 503,
 505, 511 (25), 531
 Caton, W L, 501

- Cavanaugh, M. W., 16 (148), 17 (146), 27, 495 (193), 506
 Cavazos, L. F., 34 (13), 55
 Cedard, L., 117 (238), 153
 Cesa, J., 248 (42), 261
 Chaikoff, I. L., 387 (67), 388 (67), 395, 498 (110, 200), 504, 507, 547 (90), 591
 Chalmers, J. R., 582 (24), 589
 Champion, L. R., 175, 181
 Chandrashaker, B., 161, 162, 161
 Chang, H. C., 526 (26, 27), 534
 Chang, M. C., 133, 148, 227, 252 (43), 261, 400 (22, 23), 429, 431, 437, 466
 Chapman, A. B., 228 (47), 229 (48), 230 (36), 261, 337 (27), 338 (45), 339 (45), 350 (45, 62), 356, 357
 Chapman, E. M., 496 (32), 502
 Chen, G., 131 (64), 149
 Cheng, C., 195 (43), 217
 Chester-Jones, I., 190 (155), 220
 Chow, B. F., 78 (23), 93 (74), 96 (74), 106, 108, 197 (98), 219
 Christensen, G. C., 50 (14), 51, 55
 Christian, J. J., 174 (97), 182
 Christian, R. E., 251 (49, 177), 252 (18), 260, 261, 265
 Christiansen, E. C., 117, 146
 Chu, J. P., 125 (40), 148, 166, 170, 181
 Chung, A. C., 579 (146), 580 (25, 146), 581 (146), 582 (146), 589, 593
 Cilotti, R., 232 (35), 261
 Claesson, L., 19 (22), 24, 76 (25), 82 (25), 106
 Clapp, H. A., 229, 261
 Claringbold, P. J., 117 (112), 131 (112), 136 (108), 150, 152, 319 (38), 336
 Clark, A. J., 531 (34), 534
 Clark, C. F., 230 (51), 261
 Clark, G., 209 (44), 210, 217
 Clark, P. J., 40 (45), 56, 133, 151
 Clark, R. T., 294 (16), 298 (10, 17), 299 (16), 330
 Clark, S. L., 94 (72), 169
 Clark, W. E. L., 169 (46), 209 (45), 217
 Clarke, P. M., 548, 559
 Clayton, G. W., 116 (41), 149
 Clegg, M. T., 22 (23), 24, 88 (26), 106, 116 (41a), 148, 208 (47), 209, 217, 323 (129), 333, 455, 467, 498, 502, 519 (79), 521 (79), 529 (28), 534, 535
 Clemente, C. D., 207 (96), 210, 219
 Cleveland, R., 213 (202), 221, 347, 355
 Cloette, J. H. L., 457 (14), 462 (14), 464, 485, 466, 467
 Coburn, F. D., 519 (29), 534
 Cochrane, R. L., 515 (30), 534
 Coffin, D. L., 72 (27), 106
 Cohen, S. H., 254 (100), 262
 Cohen, S. L., 502
 Cole, C. L., 292 (134), 333
 Cole, H. H., 22 (23), 24, 34, 55, 77 (31), 81 (28, 33), 82 (80), 88 (26, 29, 30, 32), 89, 96 (32), 106, 107, 108, 116 (41a), 143 (42, 80), 148, 149, 236 (52), 237 (52), 238 (52), 239, 241 (52), 251, 254, 261, 263, 271, 282 (25), 287, 288, 294 (19, 20, 21), 296, 297 (21), 298 (21), 299 (21), 300 (21), 304 (21), 310 (18, 19), 311 (18), 312, 313 (19), 327, 330, 341 (28), 353, 355, 356, 364, 373 (37), 374 (37), 378 (37), 379, 380 (37), 382 (37), 383, 394, 455, 467, 470 (35), 472 (139), 473 (40), 474 (83), 462 (41), 488, 492, 493, 494 (34), 495 (39), 497 (37), 498 (32a, 37, 39), 490 (27), 502, 505, 555 (15), 589
 Cole, R. D., 76 (35), 107, 544 (91), 545 (91), 546 (91), 562 (91), 591
 Collins, E. J., 193 (190), 213 (191), 221
 Collins, V. P., 175 (135), 176 (135), 183
 Collip, J. B., 77 (36), 107, 117 (9), 137 (224), 148, 153, 475 (168), 482 (41), 483 (41), 492 (42), 502, 506
 Combescot, C., 120 (140), 151
 Conline, R. S., 479 (12), 487 (12), 502
 Comstock, R. E., 2 (150), 3 (150), 5 (150), 7 (150), 8 (150), 9 (150), 16 (150), 27, 436 (82), 444 (62), 447 (82), 462 (82), 463 (62), 466 (62), 469
 Comte, L., 475, 502
 Conway, C. H., 31, 55
 Cont, D. N., 48, 56

- Constantinescu, G. K., 272, 273, 276 (26), 288
- Contopoulos, A. N., 90 (37, 38), 103 (37), 104 (37, 38), 107
- Cook, A. C., 226 (195), 265
- Coppen, F. M. V., 242 (22, 23), 260
- Corner, G. W., 19 (24), 21 (25), 22 (6), 23, 24, 341, 343, 345, 346, 349 (19), 355, 356, 453 (16), 454 (16), 467, 472 (46), 473 (44, 46), 490 (46), 491 (45), 496 (32), 502
- Cornwell, W. S., 345, 356
- Cortez, E., 518 (32), 534
- Coste, F., 118 (43), 148
- Cotchin, E., 392, 394
- Cotes, P. M., 579, 580 (26), 581 (26), 589
- Courrier, R., 120 (44), 131 (45), 149, 174 (27), 180, 450 (17), 467, 473 (47), 474 (47), 478 (47), 479 (47), 503
- Cowart, F. E., 270 (14), 277 (12, 14), 286 (12), 287
- Cowdry, E. V., 29 (18), 56
- Cowie, A. T., 81 (39), 107, 139 (46), 149, 202 (49), 215, 217, 472 (50), 476 (50), 479, 491 (16, 51), 502, 503, 540 (9, 32), 541 (9, 10), 542 (9, 10, 32), 543 (9), 546 (30), 547 (30), 554 (27, 29, 33), 555, 558 (31), 559 (31), 564 (30), 565 (30), 568 (9), 589, 590
- Cowie, D. B., 526 (49), 535
- Cowles, R. B., 39 (19), 56
- Cowsert, W. C., 584 (130), 592
- Cox, C. P., 491 (16), 502, 540 (9), 541 (9, 10), 542 (9, 10), 543 (9), 568 (9), 589
- Cozens, D. A., 90 (40), 104 (40), 107
- Craig-Bennett, A., 120 (47), 149
- Crampton, E. W., 582 (14), 583 (14), 584 (14), 589
- Crew, F. A. E., 38 (20), 56, 166, 180
- Crichton, J. A., 579 (26), 580 (20), 581 (26), 589
- Critchlow, R. V., 195 (50), 201, 217
- Cross, R. A., 216 (52, 53), 217, 253 (51), 259, 261, 558, 559, 590
- Cuipo, A., 346, 355, 472 (46), 473 (46), 490 (46), 502, 529 (33), 531
- Cummings, J. N., 272, 274, 275, 276, 288
- Cupps, P. T., 144 (48), 149, 172, 174, 180, 238, 245, 246, 249, 261
- Curson, H. H., 464, 465, 467, 468
- Curtis, G. M., 32 (21, 43), 56
- Curtis, Q. F., 349, 355
- Cuyler, W. K., 69 (167), 110, 125 (243, 244), 128, 152, 153, 154, 197 (198), 221
- Cuypers, Y., 130 (24), 148
- D
- Daily, W. J. R., 197, 217
- Dalcq, A. M., 415, 422, 424, 426, 431
- Dale, H. H., 177, 180
- D'Amour, F. E., 387 (58), 389 (58, 59), 390 (59), 295
- Danforth, D. N., 21 (26), 24
- Daniel, P. M., 195 (55, 56), 217
- Dantchakoff, V., 135 (49, 50), 149
- Danziger, S., 22 (149), 27
- Darlow, A. E., 294 (13), 297 (13), 300 (62), 310 (10), 323 (11), 329, 330, 331, 473 (15), 502
- Danzier, L., 178 (33), 180, 294 (23), 299, 312 (26), 313 (23), 325 (23, 26), 326 (24), 329, 330
- Davenport, N., 294 (139), 333
- David, H. A., 413 (16), 431
- Davidson, J. M., 197, 217
- Davies, D. V., 48 (65), 57
- Davies, J., 436, 447, 467
- Davis, H. P., 544 (134), 592
- Davis, M. E., 62 (41), 107, 478, 479, 496 (52), 497, 503
- Davis, R. E., 226 (195), 265
- Davison, W. F., 279 (28), 280 (28), 285
- Dawson, A. B., 19 (27), 21, 405 (25), 431
- Day, B. N., 353, 356
- Day, F. T., 269 (30), 270 (30), 278 (30), 279, 280 (29, 30), 282 (31), 290 (31, 32, 39), 288, 455, 467, 488, 498 (53), 503, 566 (68), 568, 577, 591
- de Alba, J., 225 (7), 234 (7), 236 (7), 238 (7), 241 (5, 7), 245 (5), 246 (5), 247 (5), 253 (5), 260

- Deane, H. W., 20 (28), 21 (28), 24, 493 (100), 504
 Deanesly, R., 20 (29), 24
 De Boer, S., 531 (34), 534
 Deese, R., 251 (178), 265
 de Groot, J., 207 (98), 210, 219
 de Jongh, S. E., 79 (125), 104 (124, 125), 109, 134, 151
 Delabarre, F., 118 (43), 148
 De Lange, M., 238 (57), 261
 Demmel, M., 391, 394
 Dempsey, E. W., 138 (51), 149, 206 (59), 208, 217, 218, 448, 447 (87), 450, 453 (87), 454, 481, 468
 Dennison, M., 44 (53), 56, 122 (148), 151
 De Robertis, E. D. P., 402, 431
 De Snoo, K., 525 (35), 534
 DeVita, J., 391 (31), 394
 de Vos, D., 298 (109), 332
 Dey, F. L., 198 (81), 197, 198, 208, 210 (33), 214 (83), 217, 218, 531 (36), 534
 Deysach, L. J., 54 (22), 56
 Dicker, S. E., 530 (37), 531, 534
 Dickerson, G. E., 34 (40), 56, 337 (55), 350 (55), 358
 Dickson, G. T., 582 (24), 589
 Diezfulusy, E., 90 (42), 94 (42, 43), 107, 136, 149
 Dietel, H., 20 (30), 24
 Dinerstein, J., 147 (82), 149
 di Fillo, F., 74 (158), 110
 Dix Aronald, P. T., 565 (93), 568 (93), 569 (93), 591
 Dodd, J. M., 130 (55), 149
 Dodds, E. C., 34 (23), 56
 Doisy, E. A., 131, 133 (241), 135, 148, 153, 529 (99), 536
 Domm, L. V., 170, 180
 Donahue, J. K., 130 (56), 149
 Donald, H. P., 463 (21), 467
 Donaldson, H. H., 457 (22), 463 (22), 465 (22), 467
 Donker, J. D., 582 (36), 590
 Donovan, B. T., 188, 190, 201 (68), 213 (68a), 218, 256, 261
 Dorfman, R. I., 29, 34 (25), 46 (24), 56, 113 (63), 114, 116, 117 (59), 120, 121, 124, 125 (143), 128, 129, 135, 130 (60), 147, 149, 151, 480 (57), 481 (56, 57), 484 (57), 485 (57), 503
 Dortzbach, C., 213 (189), 221
 Dott, N. M., 209 (45), 217, 386 (32), 394
 Dowling, D. F., 252 (59), 253 (59), 261
 Dracy, A. E., 248 (75), 262, 474 (78), 500 (78), 501 (160), 503, 506, 532 (58), 535
 Dragstedt, L. R., 555 (37), 590
 Draper, R. L., 484 (23), 467
 Drescher, J., 17 (128), 26, 89 (44), 107
 Dreyer, N. B., 531 (34), 534
 Drum, G. M., 463 (32), 467
 Dry, F. W., 517 (39), 534
 Dudley, F. J., 513 (159), 517 (159), 537
 Duff, V. B., 478 (20), 502
 Dukes, H. H., 267, 288
 du Mesnil du Buisson, F., 178 (33), 180, 353, 356
 Dun, R. B., 304 (75), 310 (75), 325 (75), 327 (75), 331
 Dutt, R. H., 188, 180, 255 (60), 261, 294 (32), 311 (30, 31, 33), 312 (30), 313 (31), 325 (30, 31), 328 (33), 327 (33), 330, 471 (59), 503
 Duval, M., 437 (24, 25, 28), 441 (24, 25, 28), 450, 459 (24), 460 (24, 25, 26), 462 (24, 25, 26), 467
 du Vigneaud, V., 78 (45), 107
 Dvoskin, S., 79 (46), 107
 Dykshorn, S. W., 547 (139), 592
 Dyrenfurth, I., 487 (190), 506
- ### E
- Eaton, O. N., 34 (93), 57, 328 (34), 330, 574, 590
 Ebling, F. J., 115 (65), 149
 Eckles, C. H., 463 (27, 28), 467
 Eckstein, P., 2 (31, 32), 24, 141, 143 (67), 145, 149, 267 (35), 268 (35), 274 (35), 278 (34), 280 (35), 283, 288, 378 (33), 393 (33), 394
 Eden, E. L., Jr., 390, 396
 Edgar, D. G., 233 (81), 253, 261, 308, 309, 330, 529 (40), 534
 Edgar, R. D., 140 (68), 149
 Edmondson, J. H., 228 (98), 229, 262
 Edwards, J., 229 (64), 261

Edwards, R. G., 82 (67), 108
 Ehrenberg, R., 526 (41), 534
 Ehrich, W. E., 174 (97), 182
 Eichelberger, L., 123 (136), 151
 Eichner, E., 20 (33), 24
 Elden, C. A., 344, 355, 356
 Elftman, H., 33, 56, 472 (10), 502
 Elias, J. L., 554, 590
 Elkin, D. C., 527 (42), 534
 Elks, J., 582 (24), 589
 Ellenberger, W., 2 (34), 24, 29 (27),
 56, 369, 394, 457, 467
 Elliott, G. M., 559 (40), 582 (40), 590
 Elliott, J. R., 542 (41), 550 (41), 551
 (41), 590
 Ellis, R. A., 22 (151), 27
 Ellison, E. T., 122, 149
 El-Sheikh, A. S., 298, 330
 Ely, F., 590 (42), 558, 590
 Emmel, V. E., 36 (49), 56
 Emmens, C. W., 115 (196), 117 (79),
 121 (96), 126 (70, 72, 76, 77, 78),
 128, 129 (33), 133, 134, 135, 136
 (72), 148, 149, 152, 319, 330
 Emerson, M. A., 226 (127), 244 (127),
 263
 Enders, R. K., 514 (112), 536
 Eness, P. G., 226 (127), 244 (127), 263
 Engel, E. T., 470 (172), 506
 Engel, M. B., 489 (30), 502
 Engelbregt, A., 79 (125), 109
 Engle, E. T., 34 (57), 43 (28), 56, 163,
 180, 384, 394
 Engstrom, W. W., 170 (08), 181
 Epstein, M., 34 (60), 57
 Erb, R. E., 253 (74), 262
 Erdheim, J., 475, 503
 Eriksen, S., 374 (36), 391 (30), 394
 Ershoff, B. H., 87 (47), 107
 Eschricht, D. F., 440 (30), 467
 Evans, E. I., 245, 246 (65), 262, 530
 (43), 531
 Evans, H. M., 68 (57), 69 (139, 141,
 142), 71 (139, 141, 142), 72 (58),
 74 (55, 56, 57, 69, 142), 70 (98,
 99, 101), 77 (52), 83 (21), 87 (48,
 118, 119, 120, 121, 174), 88 (51,
 53, 99), 89, 90 (52, 54), 91 (55,
 50, 58, 102), 92 (52, 55, 58, 110),
 93 (58, 140), 97 (49), 99 (52),

101 (54), 103 (49, 52, 58, 68),
 105 (68), 106, 107, 108, 109, 143
 (80, 159), 144 (159), 149, 151,
 364, 369 (38), 370 (38), 373, 374
 (37), 378 (37), 379, 380 (37), 382
 (37), 383, 385 (38), 387 (37a),
 394, 395, 472 (62), 487, 496 (75),
 503, 516, 536

Evans, R. D., 496 (32), 502
 Evans, T. C., 496 (96), 504
 Everett, J. W., 80 (59), 107, 192, 200
 (142, 170), 201, 202 (70), 217,
 218, 220, 255 (124), 256 (158),
 257 (158), 263, 264
 Everett, N. B., 550 (8), 589
 Eversole, W. J., 554 (62), 590
 Ewart, J. C., 437 (31), 441 (31), 445
 (31), 450 (31), 455 (31), 467

F

Faiermark, S. E., 348, 352, 356
 Farris, E. J., 100, 107
 Fawcett, D. W., 34 (29), 56
 Fazekas, J. F., 554 (123), 592
 Fedor, E. J., 193 (190), 213 (191), 221
 Fee, A. R., 34 (30), 56, 144, 149, 198
 (75), 199 (75, 76), 218
 Fekete, K., 531 (44), 534
 Fellner, O. O., 130 (82), 149
 Fels, S. S., 124 (228), 153
 Ferguson, J. K. W., 214, 218, 219, 530
 (45, 64), 534, 535
 Ferner, H., 20 (30), 24
 Fernstrom, I., 21 (9), 23
 Ferrante, L., 529 (10), 534
 Fevold, H. L., 67 (02), 80 (01), 82
 (83), 107, 108, 200, 218, 257 (06),
 262, 472 (9), 502, 530 (07), 536
 Fichera, G., 122 (83), 149
 Fieblger, J., 2 (131), 26, 29 (69), 32
 (89), 38 (89), 42, 44, 48 (89),
 51 (89), 57, 369 (103), 370, 371
 (103), 383 (103), 396
 Finerty, J. C., 70 (63), 107, 528 (40),
 531
 Finkelstein, M., 137 (84), 150
 Finney, D. J., 150 (65), 150
 Fisher, C., 197 (02), 198 (62), 211
 (03, 79), 218, 531 (30), 534
 Fisher, G. T., 497 (98), 501

- Fitch, J. B., 163 (101), 164 (101), 182, 250 (166), 264, 463, 467
- Fitzpatrick, R. J., 179, 180, 205 (80), 218, 530, 534
- Fleming, A. M., 527 (46), 535
- Flexner, L. B., 528 (49), 535
- Flocks, R. H., 46 (31), 58
- Flux, D. S., 540 (9), 541 (9), 542 (9), 543 (9), 545, 566 (9), 561 (44), 589, 590
- Foa, C., 213, 218
- Foley, R. C., 2 (35), 22 (35), 24, 174, 181, 234, 262
- Folley, S. J., 61 (39, 84), 107, 125 (16), 132, 134 (86), 139, 146, 149, 150, 202 (49, 82), 215, 217, 216, 242 (22, 23), 253 (68), 256, 260, 262, 472 (50), 476 (50), 477 (64), 476, 479 (65), 491 (51, 64), 503, 540 (9, 32), 541 (9), 542 (9, 32), 543 (9), 546 (30, 47), 547 (30), 546 (46, 46), 550 (47), 553 (11, 12), 554 (29, 49), 555, 556 (31), 559 (31), 580 (49), 564, 565 (30, 49, 51), 566 (50, 51, 54), 566 (9), 577, 579, 580 (26), 581 (26, 44, 56), 582 (7, 49), 584 (55), 586 (45), 589, 590
- Follis, R. H., 87 (85), 108
- Fomenko, M. V., 304 (104), 329 (105), 332
- Foot, R. H., 228, 263
- Foot, W. C., 337, 358
- Foot, W. D., 501 (66), 503
- Forbes, T. R., 11 (36), 24, 84 (66), 108, 118, 150, 308, 331, 485, 486, 504, 529, 535
- Ford, D. H., 23 (37), 24
- Fortgang, A., 530 (51), 535
- Fortier, C., 195 (83, 84, 65), 218
- Fontaine, F. C., 586 (20), 589
- Fowler, R. E., 82 (67), 108
- Fraenkel-Conrat, H., 74 (69), 103 (68), 105 (88), 108
- Frahm, H., 89 (137), 109
- Fralick, R. L., 18 (147), 27
- Frank, A. H., 253 (176), 265, 310 (40), 313 (40), 328 (102), 330, 332, 408 (27), 431
- Frank, R. T., 529 (52), 535
- Frankenbach, R. F., 163 (24), 180, 250 (33), 261
- Franklin, K. J., 524 (53), 535
- Fraps, R. M., 249, 255, 262, 326 (102), 332
- Frazier, J. D. F., 475 (67), 503
- Frazier, C. N., 568 (58), 590
- Fredeen, H. T., 168, 181
- Fredrickson, D. S., 196 (69), 197 (89), 218
- Freud, J., 134 (66), 150
- Freusberg, A., 527, 535
- Frey, E., 189 (86), 218
- Frick, E. J., 391 (39), 395
- Fridhandler, L., 429 (28), 431
- Fried, P. H., 96 (70), 108, 526 (163), 537
- Frieden, E. H., 146 (69), 150, 474 (68), 503
- Friedgood, H. B., 193 (87), 218, 405 (25), 431
- Friedman, M. H., 136 (181, 162), 151, 392 (40), 395
- Friley, M., 16 (114, 115), 17 (115), 26
- Froesch, E. R., 216
- Fromageot, C., 530 (2), 533
- Fry, E. G., 198 (140), 216, 220
- Fry, R. M., 246 (154, 155), 264
- Frye, J. B., Jr., 226 (31), 261
- Fueffel, C., 462 (63), 464, 466, 466
- Fugo, N. W., 125 (250), 154
- Fukuda, M., 202 (185, 186, 187), 221, 258 (161, 162, 163), 264
- Funk, E. M., 189 (23), 180

G

- Gabuten, A. R., 169 (38), 181
- Gaines, W. L., 558, 590
- Gallagher, T. F., 34 (25), 56, 123 (183), 128, 150, 152
- Gallien, L., 127, 135, 150
- Gallimore, E. J., 34 (23), 56
- Canong, W. F., 198 (89), 197, 208 (47), 209 (47, 48), 213, 217, 218, 323 (129), 333
- Gardner, W. U., 133 (93), 150, 389 (41), 395, 477 (69), 503, 542 (81), 547 (80), 560, 566 (157), 590, 592, 593
- Garm, O., 174, 181, 242 (71), 249, 262

- Garrigan, V S, 501 (197), 507
 Garnigus, U S, 295 (149), 333
 Gaunt, R, 554 (62, 122), 590, 592
 Gebhard, H, 206 (128), 219
 Gee, W, 367 (5, 6), 393, 394
 Geist, S H, 127 (222), 153
 Gellert, R, 213, 218
 Gerlaugh, P, 512 (123), 513, 535, 536
 Gerlinger, H, 370 (42), 378, 395
 Gersh, I, 446, 467, 472 (29), 473 (71),
 475 (70), 494 (29, 71), 495 (29),
 497 (72), 502, 503
 Gershon Cohen, J, 124 (228), 153
 Gey, C O, 88 (71), 108, 493 (73, 99),
 503, 504
 Gey, M K, 493 (99), 504
 Gbanem, Y S, 307 (41), 330
 Gier, H T, 364 (78), 366 (43), 374,
 380, 395, 396
 Gill, W M, 580 (25), 589
 Gillman, J, 3 (38), 4, 24, 142 (131),
 151
 Gilmore, R, 365, 394
 Ginsburg, N, 140 (211), 153
 Gioia, J D, 21 (78), 25
 Glazener, E W, 167, 181
 Glenister, T W, 2 (39), 24
 Gley, E, 124, 148
 Gloor, P, 210 (90), 218
 Glover, F A, 242, 260, 262, 490 (74),
 503
 Godfrey, G F, 169 (41), 180
 Goetze, R, 268 (37), 288
 Goldberg R C, 196, 218
 Goldfien, A, 218
 Goldstein, A C, 205 (93), 206, 210,
 211 (93), 218
 Goldstein, S R, 454, 467
 Goldzweig, S A, 491 (16), 502, 541
 (10), 542 (10), 589
 Goltz, F, 527, 535
 Gomez, E T, 383, 388 (105), 396, 554
 (63), 590
 Gomori, G, 257 (73), 262
 Gooch, L D, 501 (66), 503
 Goodman, L, 202 (94), 207 (94), 218
 Goodman, L S, 195 (43), 217
 Goodrich, F J, 120 (118), 150
 Goodwin, M P, 34 (34), 56
 Goodwin, W E, 34 (10), 55
 Gorbman, A, 97 (49), 103 (49), 107,
 496 (75, 75a), 503
 Gordis, L, 74 (158), 110
 Gordon, I, 325 (43), 326 (42), 330
 Gordon, W B, 175 (135), 176 (135),
 183
 Gorski, J, 253, 262
 Goss, H, 88 (29, 30), 106, 107, 455,
 467, 493, 497 (37), 498 (37), 502
 Goto, T, 566 (64), 590
 Gould, R G, 497 (53), 503
 Gow, R S, 169 (57), 181
 Gowen, J W, 555 (65), 590
 Graham, C L, 167 (127), 183
 Graham, E F, 246 (75), 262, 474 (76),
 500 (76), 503, 532 (56), 535
 Graham, M A, 2 (40), 24
 Grant, F G, 212, 221
 Grant, R, 294 (44), 295 (44), 297 (44),
 300 (44), 304 (44), 330, 457, 467
 Grau, H, 29 (94), 51 (94), 57
 Gray, H, 41 (32), 56
 Green, J D, 194 (95), 207, 210, 218,
 219
 Green, S H, 19 (41, 42), 24
 Green, W W, 2 (43, 150), 3 (150), 5
 (150), 7 (150), 8 (150), 9 (150),
 10 (150), 24, 27, 34 (12), 55, 298
 (45), 299 (45, 48), 330, 438, 444
 (82), 447, 462, 463 (36), 485 (82),
 466 (82), 467, 468
 Greenbaum, A L, 81 (64), 107
 Greenblatt, R B, 94 (72), 108, 127
 (94), 150, 387 (44), 389 (44), 395
 Greene, E G, 43 (33), 56
 Greene, E G, 22 (119), 26
 Greene, H S N, 221, 518 (131), 537
 Greene, R R, 2 (97), 25, 127 (97), 128,
 137, 150
 Greenstein, J S, 174, 181, 234, 262
 Greenwood, A W, 34 (23), 56, 128,
 150, 166, 170, 181
 Greep, R O, 2 (44), 24, 75 (5), 76
 (23), 78 (85), 79 (73), 82 (83),
 93 (74), 98 (74), 106, 108, 193,
 197 (98), 200 (78), 218, 219, 257
 (66), 262
 Greer, M A, 196, 199, 219, 221, 477
 (77), 493 (77), 503
 Gregoire, G, 554 (60), 555 (60), 590

- Gregory, P. W., 429 (36), 432, 520, 522 (57), 535
 Grenlich, W. W., 144 (101), 150
 Griffin, S. A., 158 (52), 160 (52), 181
 Griffiths, W. F. B., 364 (45), 374, 379 (45), 380 (45), 392, 395, 423 (2), 424 (2), 426 (2), 431, 437 (38), 450 (36), 467
 Grobstein, G., 124 (102), 150, 190 (101), 219
 Grollman, A., 497 (72), 503
 Groome, J. R., 31, 43 (96), 46 (96), 47, 57
 Gros, C., 450 (17), 459 (39), 467
 Grosser, O., 434 (40), 435, 444 (40), 451, 467
 Grossman, J. D., 2 (123), 26, 29 (87), 41 (87), 42 (87), 57, 229 (164), 264
 Grosvenor, G. E., 259 (78), 262, 552 (67), 554, 555 (67), 590 (67), 590
 Grummer, R. H., 337 (27, 49, 82), 338 (45, 49, 62, 66), 339 (45), 340 (6, 81), 341 (6), 350 (45, 49, 62), 351 (49), 352 (58), 353 (60), 354 (5, 34), 355, 356, 357
 Grünwald, P., 2 (47), 3, 4, 11 (45, 46), 24
 Grueter, F., 547, 593
 Grunt, J. A., 206, 219
 Guilbert, H. R., 226 (77), 262
 Guillemin, R., 201 (103), 219
 Gunther, M., 530 (58), 535
 Gustavson, R. G., 387 (58), 369 (58, 59), 390 (59), 395, 529 (52), 535
 Guthrie, M. J., 297 (80), 331
 Gutteridge, H. S., 169 (44, 45), 181
 György, P., 497 (191), 506

H

- Hadek, R., 21 (48), 24, 298 (47, 48, 49, 50), 330, 430 (29), 431
 Hafez, E. S. E., 292 (51), 293, 294 (51), 330, 429 (28), 431
 Hagopian, M., 492 (78), 494 (78), 503
 Haines, W. J., 34 (34), 56
 Hair, G. W., 193, 219
 Halama, A., 354 (37), 356
 Halasz, B., 288 (38), 288

- Hall, B. V., 424 (44), 432
 Hall, J. C., 226 (31), 261
 Hall, K., 123 (150, 152), 126 (149, 151), 146, 147, 150, 151, 532 (59), 535
 Hall, N. M., 168 (113), 169 (113), 182
 Hall, S. R., 574 (38), 590
 Hallgren, W., 520 (60), 535
 Halmi, M. S., 192 (31, 32), 198 (31, 32), 197 (31), 217
 Halnan, E. T., 382 (66), 395
 Ham, A. W., 29 (35), 56
 Hamburger, C., 77 (31), 90 (75, 76), 96 (75), 97 (78a), 100 (75), 107, 108, 122 (106), 150
 Hamilton, J. B., 34 (71, 72), 57, 115 (37, 109), 122 (37, 110), 123 (107, 108), 148, 150
 Hamilton, W. F., 524 (185), 538
 Hamilton, W. J., 279, 286 (39), 288, 329, 331, 373 (22), 394, 400 (17), 422, 423 (2), 424, 428, 431
 Hamlett, G. W. D., 90 (77), 108
 Hammond, J., 22 (48a), 24, 142 (245), 144 (111), 150, 154, 179, 181, 234 (79), 239, 241 (79), 258 (78), 262, 268 (41, 43, 45), 271 (41, 43, 45), 274, 276 (45), 277, 278 (43, 45, 48), 279 (45), 280 (42, 46), 282, 283, 284, 288 (43, 44, 45), 267, 288, 292 (55), 294 (54), 297 (53), 310 (56), 311 (56), 312 (58), 326 (56), 331, 372 (46), 395, 437, 444 (47), 445 (42), 448, 457 (42), 458, 462 (42), 465, 467, 491 (79), 503, 513 (61), 517 (162), 535, 537, 568 (68), 568, 577, 591
 Hammond, J., Jr., 187, 188, 189, 190, 191 (106), 219, 252 (80), 253 (80), 262, 294 (56), 297 (56), 310 (58), 311 (57, 58), 312, 328 (58), 331
 Hamperl, H., 22 (49), 24
 Hancock, J., 563, 569, 560 (22), 581 (22), 589, 591
 Hancock, J. L., 272, 288, 362 (47), 364 (47a), 379 (47), 380 (47), 382 (47), 384 (47), 389 (47, 47b), 391, 392 (47), 393 (47), 395
 Hanka, L. J., 228 (127), 244 (127), 263

- Hansel, W, 224 (167), 225 (103, 167),
 226 (103), 227 (84, 85, 89, 103,
 167), 228, 230 (12, 167), 231
 (83, 167), 232 (91), 233, 234
 (167), 230 (167), 238, 241, 244
 (81), 245 (83), 246 (81, 82), 247
 (89), 249 (89, 91), 251, 253, 254
 (91), 255, 258, 260, 262, 264, 265,
 303, 331, 472 (80), 503
 Hansson, A, 224, 262
 Haour, P, 96 (157), 110
 Harada, N, 270 (67), 289
 Hardy, M M, 117 (112), 131 (112),
 150
 Harkness, M L R, 490 (81, 82), 504
 Harkness, R D, 490 (81, 82), 504
 Harper, G, 577 (4), 589
 Harns, C W, 188, 193, 194, 195, 196
 (109), 199, 203, 204, 209, 213, 215,
 218, 219, 255, 256, 261, 262, 386
 (48), 395, 527 (62), 535, 559, 590
 Harris, J, 473 (86), 504
 Harris, J W, 216 (53), 217
 Harrison, R G, 31 (36), 35, 36 (36,
 37), 56
 Harrison, R J, 22 (50), 23 (50), 24,
 142, 150, 280 (48), 288, 329, 331
 Harrop, A E, 392 (49), 395
 Harrop, C A, 387 (101), 396
 Hart, D S, 188, 219, 292 (60), 331
 Hart, C H, 34 (15), 55, 88 (32), 96
 (32), 107, 310 (18), 311 (18), 312
 (18), 330, 359 (7), 394, 474 (83),
 488 (38), 492, 495 (39), 498 (39),
 502, 504
 Hartig, F, 40 (39), 56
 Hartman, C G, 2, 18 (53), 19 (24), 22
 (6, 51), 23, 24, 82 (78), 96 (78),
 108, 143 (114), 150, 178, 181, 370
 (50), 392 (50), 395, 406 (31), 431
 Haskins, A L, 140 (115), 150, 254
 (94), 262, 528 (63), 535
 Hatai, S, 122 (116), 130 (116, 117),
 150
 Hatch, R D, 22 (54), 24
 Hatch, R N, 500 (129), 505
 Haterius, H O, 139 (191), 152, 214
 (116), 219, 474 (84), 504, 529
 Hathaway, I L, 544 (134), 592
 Hauser, E R, 34 (40), 56, 294 (13),
 297 (13), 330
 Hawk, H W, 224, 262
 Hawker, R W, 531 (65, 66), 535, 558
 (70), 591
 Hawkins, L E, 294 (13), 297 (13),
 300 (62), 330, 331
 Haymaker, W, 189 (117), 219
 Hays, E E, 76 (79), 108
 Hays, F A, 167, 181
 Hays, H W, 128 (172), 152
 Hays, R L, 178, 179, 181, 183, 245,
 246, 247, 257, 262, 265, 407 (56,
 57, 58), 432
 Hazel, L N, 353 (22), 356, 511 (150),
 513 (150), 515 (150), 517 (150),
 519, 537
 Hazleton, L W, 120 (118), 150
 Heape, W, 141, 150, 295, 331, 360,
 364, 383, 384, 395
 Heard, R D H, 114 (120), 150
 Hechter, O, 471, 473 (86), 487 (87),
 504
 Heckel, C P, 529 (67), 535
 Heims, B A, 582 (24), 589
 Hemncus, G, 437 (44), 442 (44), 445
 (44), 458 (44), 459 (44), 460
 (44), 461 (44), 467
 Heitman, H, Jr, 82 (80), 108, 341
 (28), 353, 356
 Hellbaum, A A, 132, 152, 173, 180
 Hellman, L M, 88 (71), 108, 493 (73),
 503, 526 (49), 535
 Hellweg, C, 22 (55, 56), 24
 Helm, K, 382 (53), 395
 Hemmings, W A, 429 (20), 431
 Hench, P S, 173 (102), 174 (102), 182
 Henderson, C R, 225 (145), 264
 Henderson, V E, 204 (118), 219
 Henderson, W R, 385 (88), 387 (88),
 390 (88), 396
 Hendricks, S B, 187, 219
 Henneman, H A, 158 (52, 88), 160,
 181, 182
 Hensen, V, 142 (122), 151
 Herbert, J, 188, 209, 219
 Herlant, M, 138, 151, 475 504
 Herhgoeffer, K M, 21 (57), 24

- Herlihy, W. F., 42, 56
 Herman, H. A., 225 (150), 228 (98),
 229, 234 (150, 187), 236, 238
 (150), 239, 242, 243, 262, 264, 265,
 584 (130), 592
 Herrick, E. H., 175, 181
 Herrick, J. B., 239, 262
 Hertz, R., 87 (81, 82), 108
 Hesselberg, C., 544 (88), 562 (88), 578
 (88), 591
 Hestrin, S., 137 (84), 150
 Hettig, R. A., 175 (135), 178 (135), 183
 Heuser, C. H., 2, 25, 424 (32), 432, 436
 (45), 440, 446 (45, 46), 447 (45),
 453 (45), 454 (45), 467
 Heyde, M., 528, 537
 Hibbs, J. W., 555 (71), 591
 Hill, D. L., 254 (100), 262
 Hill, M., 145 (124), 151, 188 (121), 219
 Hill, R. T., 472, 476 (90), 504
 Hullarp, N. A., 198, 210, 219
 Hinsey, J. C., 468
 Hinterthur, W., 517 (89), 535
 Hinwich, H. E., 554 (123), 592
 Hirsch, E. F., 527 (21), 534
 Hisaw, F. L., 67 (82), 79 (136), 82
 (83), 107, 108, 109, 132 (155),
 140 (220), 148 (89, 125, 126, 127),
 147 (127), 150, 151, 153, 173, 174,
 181, 200 (78), 218, 257 (66), 262,
 345, 356, 473 (93), 474, 483 (94),
 485 (94), 489 (95), 501 (95), 503,
 504, 530 (97), 532, 535, 536
 Hisaw, F. L., Jr., 473 (183), 506
 Hoar, W. S., 120 (128, 202), 151, 152
 Hodges, C. V., 123 (136), 151
 Hodges, R. E., 496 (96), 504
 Högberg, B., 76 (25), 62 (25), 106
 Hohn, E. O., 541 (72), 545, 591
 Hoffman, E., 167 (127), 169, 161, 183
 Hoffman, F., 116, 151
 Hoffman, J., 2 (59), 25
 Hoflinger, H., 231, 262
 Hofstad, M. S., 229 (102), 262
 Hogan, A. C., 169 (95), 182
 Hohlweg, W., 80 (84), 108, 198 (124),
 219
 Holcombe, R. B., 176, 161
 Hollandbeck, R., 336 (30), 356
 Hollinshead, W. H., 109, 200 (171),
 220, 255 (122), 263
 Holm, L. W., 519 (79), 521 (79), 535
 Holmstrom, E. C., 532 (168), 538
 Holz, A. M., 207 (19), 216
 Homma, H., 11 (60), 12 (60), 25
 Hoogstra, M. J., 70 (125), 109
 Hooker, C. W., 34 (42), 56, 118, 139,
 151, 308, 331, 485, 486, 504, 529,
 535, 544 (73), 554 (73), 591
 Hooper, E. C., 529 (74), 535
 Horie, T., 34 (77), 57
 Horwood, R. E., 581 (99), 588 (99),
 591
 Hoshi, S., 268, 289
 Hough, W. H., 224 (167), 225 (103,
 167), 226 (103), 230 (167), 231
 (167), 234 (167), 236 (187), 255,
 262, 264
 Houssay, B. A., 215 (125), 219, 386
 (54), 395, 547 (74), 591
 Howard, E., 123 (132, 133), 151
 Howell, C. E., 270 (49, 73), 275, 278
 (17), 277 (16), 287, 288, 289, 512,
 514, 515 (75), 518, 519 (75), 520
 (20), 534, 535, 537
 Hu, C. H., 518 (131), 537
 Huber, G. C., 32 (21, 43), 56
 Hubert, G. R., 115 (37), 122 (37), 148
 Hudson, R. S., 270 (15), 280 (15), 287
 Huffman, C. F., 139 (209), 153, 544
 (101), 569 (101), 571 (136), 577
 (136), 581 (150), 591, 592, 593
 Huggett, A. St. G., 444 (47), 467, 526
 (76), 535
 Huggins, C., 34 (46), 44 (47), 46, 47,
 56, 123, 133, 151
 Hughes, E. H., 353, 355
 Hulet, C. V., 298 (39), 330
 Hume, D. M., 196 (89), 197 (69), 216
 Humphrey, G. F., 48 (46, 65), 56, 57
 Hunter, G. L., 311 (65), 326 (65), 331
 Hunter, R. H., 5 (61), 11 (61), 12 (61),
 13 (61), 25
 Hurst, W. R., 147, 153
 Hurwitt, E., 147 (1), 147
 Hutt, F. B., 169 (57), 181
 Hutton, J. B., 580, 586, 591
 Hvaton, B. P., 352, 356
 Hyett, A. R., 22 (50), 23 (50), 24

I

- bayashu, H, 258 (137), 263
 neichen, B, 519 (77), 535
 ngle, D J, 173, 181, 497 (98), 504
 ngram, D L, 19 (62), 25
 Inkster, I J, 296 (66), 331
 Irwin, M R, 167 (111), 168 (112), 182
 Ishibashi, C, 258 (137), 263
 Israel, S L, 20 (63), 25
 Itho, M, 124 (137), 151
 Ivy, A C, 127 (97), 137 (96, 98, 99),
 150

J

- Jaap, R G, 166, 171, 181
 Jablonski, W J, 532 (78), 535
 Jackson, C M, 462 (48), 464 (48), 467
 Jackson, W F, 390 (55), 395
 Jacobsohn, D, 203, 213, 219
 Jacobson, W, 429 (33), 432
 Janes, R G, 162, 181
 Janssen, A, 467
 Jasper, D E, 519 (79), 521 (79), 520
 (80), 535
 Jaynes, J, 210 (21, 22), 211 (21, 22),
 216
 Jellinek, P H, 114 (120), 150
 Jenkinson, J W, 443 (50), 448, 468
 Jennings, W E, 277, 288
 Jensen, H, 72 (58), 91 (58), 92 (58),
 93 (58), 103 (58), 107
 Johnsen, S G, 97 (76a), 108
 Johnson, F N, 162, 181
 Johnson, H H A, 338 (32), 352, 356
 Johnson, J E, 176 (75), 181
 Johnson, L E, 513, 535
 Johnson, R E, 83 (131), 109, 544 (91),
 545 (91, 92), 546 (91), 562 (91),
 591
 Johnson, R H, 34 (34), 56
 Johnson, R M, 545, 553 (77), 554, 591
 Johnston, R F, 545 (81), 591
 Jolly, H, 212, 219, 364 (69), 374, 376
 (69), 382 (69), 394 (69), 385, 387
 (69), 396
 Jonckheere, F, 360, 302 (56), 395
 Jones, G E S, 493 (99), 504
 Jones, H E H, 481 (157), 483 (157),
 506
 Jones, I C, 79 (73), 108
 Jones, R C, 225 (115), 264

- Jones, T C, 390 (96), 396
 Joranson, Y, 36 (49), 56
 Jordan, H E, 440, 468
 Jordan, R M, 501 (166), 506, 851 (82),
 591
 Jordao, L P, 512 (82, 83), 535
 Joseph, N R, 489 (30), 502
 Josimovich, J B, 493 (100), 504
 Jost, A, 16 (64, 65, 66), 17 (65, 66),
 18 (66), 25, 119 (139), 127, 151,
 495 (101, 102), 504, 531 (84), 535
 Joubert, D M, 224, 263, 513, 517 (85),
 518 (85), 535
 Jubbs, K V, 247, 256, 263
 Julian, L M, 38 (51), 46 (51), 47 (50,
 51), 48 (51), 56
 Jull, M A, 167, 171, 181
 Junge, G C A, 121 (194), 152
 Junkmann, K, 198 (124), 219

K

- Kahn, R H, 23 (67), 25
 Kaiser, I H, 1 (81), 25
 Kamm, O, 481 (126), 483 (126), 505
 Kammlade, W G, 305, 306, 307, 310
 (67), 331
 Karn, M N, 511 (86), 536
 Kassenarr, A A H, 100 (162), 110
 Katzman, P, 529 (99), 536
 Kawai, T, 202 (185, 186, 187), 221, 258
 (161, 162, 163), 264
 Keetel, W C, 496 (96), 504
 Kehl, R, 120 (140), 151
 Keiffer, H, 527 (87), 536
 Keller, K, 463 (52), 468, 495 (103),
 504
 Kelley, R B, 292 (68, 69), 294 (68,
 69), 296, 331
 Kellner, G, 19 (17), 23
 Kellner, R, 270 (51, 52), 288
 Kelly, C L, 529, 536
 Kelly, T L, 76 (152), 110
 Kempster, H L, 168 (113, 114, 115,
 116), 169 (15, 95, 113), 180, 182,
 183
 Kendall, E C, 173 (102), 174 (102),
 182, 554 (62), 590
 Kendrick, J W, 519 (79), 520 (89,
 148), 521 (79, 89, 148), 531 (89),
 535, 536, 537

- Kennedy, J. W., 297 (80), 331
 Kennedy, P. C., 520 (89, 148), 521, 531 (89), 536, 537
 Kennedy, W. P., 392 (57), 395
 Kenneth, J. R., 509, 510, 520, 538
 Kent, G. C., Jr., 209, 219
 Kern, 270 (53), 288
 Key, J. A., 453 (88), 481, 468
 Keye, J. D., 356, 356
 Kidder, H. E., 246 (19, 21), 260, 354, 358
 Kido, I., 493 (104), 504
 Kiesling, A., 328 (70), 331
 Kimura, C., 345, 356
 Kimura, J., 492, 504
 King, H. D., 463 (53), 468
 King, J. L., 346, 356
 Kinsey, A. C., 208, 219
 Kirby, A., 38, 56
 Kirkham, W. B., 514 (91), 536
 Kirkpatrick, C. M., 188 (129), 219
 Kislovsky, D. A., 464, 468
 Kitay, J. I., 212, 219
 Kitchell, R. L., 18 (88), 25, 41 (3), 55, 497 (100), 504
 Klein, M., 126 (141), 151, 208 (132), 219
 Klempman, S., 1 (69), 25
 Kliman, B., 147, 151
 Kline, I. T., 125 (143), 151
 Kline, R. F., 554 (19), 589
 Kling, A., 210, 221
 Klüver, H., 210, 219
 Klyne, W., 481 (107), 482 (107), 504
 Kment, A., 354 (37), 356
 Knapp, B., 514, 517 (92), 536
 Knaus, N., 530 (93), 536
 Knobil, E., 78 (85), 108, 190, 218, 497 (108), 504
 Knodt, C. B., 546 (126), 582 (83), 591, 592
 Knott, J. C., 517, 536
 Kober, S., 137, 151
 Koch, F. C., 34 (25), 56, 123 (183), 128, 150, 152, 495 (201), 507
 Koch, W., 137 (84), 150
 Kochakian, C. D., 115, 151
 Kodicek, E., 429 (31), 432
 Koff, A. K., 12 (70), 25, 82 (41), 107
 Kohls, C. L., 89 (50), 107, 487 (61), 503
 Kohn-Speyer, A., 122 (148), 151
 Koibuchi, E., 202 (185), 221, 258 (161), 284
 Koikegami, H., 192, 219
 Kolster, R., 459 (55), 461, 468
 Kon, T., 124 (137), 151
 Koneff, A. A., 87 (86), 90 (38), 104 (38), 107, 108, 199 (195), 221, 496 (110), 504
 Korenchevsky, V., 44 (53), 56, 122 (147, 148), 123 (150, 152), 126 (149, 151), 151
 Koshi, J. H., 582 (36), 590
 Krallinger, H. F., 339, 341, 356
 Kraus, H., 137 (153), 151
 Krehbiel, R. H., 258 (107), 263
 Krichesky, B., 478 (111), 504
 Kritchlow, B. V., 2 (121), 26
 Krizencky, J., 517 (95), 518 (95), 519 (95), 538
 Kroc, R. L., 532 (147), 537
 Krohn, L., 473 (86), 504
 Krohn, P. L., 2 (71), 25
 Kross, I., 526, 536
 Krupski, A., 228 (108), 263
 Kubik, I., 23 (72), 25
 Kudzus, H., 126 (31), 148
 Kudzia, J. J., 175, 181
 Kupfer, M., 270 (54), 277, 278 (54), 288, 292 (71), 331
 Kuizenga, M. H., 34 (34), 56
 Kumaran, J. D. S., 168, 171, 181
 Kunde, M. M., 387, 389, 390 (59), 395
 Kunkle, L. E., 512 (123), 513 (54), 535, 536
 Kuntz, A., 204, 215 (136), 219
 Kuntz, P., 215 (30), 217
 Küpfer, M., 234 (109), 263
 Kuznecov, N. N., 352 (31), 356
- L
- Laben, R. C., 174 (36), 180, 249 (50), 261, 511 (130), 512 (130), 514 (130), 517 (130), 518 (130), 519 (130), 537
 Lacastagne, A., 134 (154), 151
 Lacroix, L. J., 382 (60), 391 (60), 395
 Ladman, A. J., 476, 493 (100), 504

- Lagerlof, N., 267, 288
 Laing, J. A., 268 (57), 274, 286 (56),
 288, 382 (61), 384, 395, 400 (35),
 426, 431, 432
 Lamas, C. H., 253 (176), 265
 Lambert, V. W., 514 (92), 517 (92),
 536
 Lambourne, L. J., 296 (72), 325 (72,
 73), 326 (142), 331, 333
 Lamming, G. E., 246 (111, 154, 155),
 252 (110), 253 (110), 263, 264
 Lamond, D., 319 (38), 321 (127), 330,
 332
 Lamont, W. A., 76 (153, 154), 110
 Lane, C. E., 132 (155), 151
 Lang, W. R., 23 (73), 25
 Langworthy, O. R., 204, 221
 Lank, R. B., 226 (31), 261
 Laplaud, M., 310 (136), 326 (74, 135,
 137), 331, 333
 Laquer, G. S., 196 (137), 220
 Larchin, B. A., 464, 468
 Larson, G. L., 228 (112), 252 (191),
 263, 265
 Lasley, E. L., 350 (39), 356, 471 (113),
 504
 Lasley, J. F., 229 (113), 263, 341 (2),
 353 (2), 355, 531 (108), 536
 Latimer, H. B., 11 (74), 25, 34 (54,
 55), 56
 Laufer, A., 72 (87), 108
 Laurent, F., 118 (43), 148
 Lawler, H. C., 78 (45), 107
 Leach, R. B., 34 (56), 56, 82 (109),
 109
 Leatham, J. H., 97 (91), 108, 380 (63),
 382 (63), 387 (62), 388, 389 (62,
 63a), 395
 Leckie, F. H. J., 20 (75), 25
 Lee, L. Y., 526 (26), 534
 Lee, M. O., 190 (138), 220
 Leech, F. B., 163, 181, 583 (84), 591
 Leibold, A. A., 391, 395
 Lemon, H. D., Jr., 176 (93), 177 (92,
 93), 182
 Leninger, C. R., 208 (64), 218
 Lensch, J., 279 (58), 288
 Leonard, E. P., 390 (65), 395
 Leonard, S. L., 67 (62), 88 (93), 90
 (92), 107, 108, 122 (156) 151, 530
 (97), 536
 Leopold, A. C., 188 (129), 219
 Leopold, G., 525 (98), 536
 Lepkovsky, S., 199 (195), 221
 LeRoy, C. V., 497 (53), 503
 Lesbouyries, M. M., 388 (66), 395
 Lesnick, G., 147 (1), 147
 Lever, J. D., 10 (76), 25
 Levin, L., 97 (91), 108, 529 (99), 536
 Levine, R., 471 (114), 504
 Levinson, G. E., 207 (20), 216
 Lewis, A. A., 566 (85, 86), 577 (86),
 591
 Lewis, L. L., 349 (40), 356
 Lewis, W. H., 429 (36), 432
 Li, C. H., 69 (141, 142), 71 (141, 142),
 74 (69, 142), 76 (35, 94, 96, 98, 99,
 100, 101, 129, 130, 150), 78 (95,
 97, 126), 88 (99, 130), 89 (129),
 91 (102), 92 (140), 93 (140), 103
 (68), 105 (68), 107, 108, 109, 110,
 494 (115), 504, 544 (91), 545 (91,
 92), 546 (91), 582 (91), 591
 Liberman, M. J., 209, 219
 Liche, H., 405 (37), 432
 Liebenow, W., 526 (41), 534
 Lillie, F. R., 15 (77), 17, 25, 409, 432,
 462 (56), 468, 495 (116), 504
 Lillie, R. J., 169 (67), 181
 Lindley, C. E., 251 (178), 265
 Lisa, J. R., 21 (78), 25
 Little, C. C., 130 (251), 154
 Liu, T. Y., 547 (87), 591
 Lloyd Davies, H., 304 (75), 310 (75),
 325 (75), 327 (75), 331
 Loeb, L., 137 (157), 138, 151, 544 (88),
 562 (88), 578 (88), 591
 Loginova, N. V., 310 (76), 326 (77),
 331
 Loke, K. H., 480 (126a), 505
 Lombard, L., 21 (79), 25, 234 (114),
 263
 Lombardo, N., 329 (1), 329
 Long, C. N. H., 76 (168), 110, 196
 (140), 218, 220
 Long, J. A., 83 (127), 109, 143 (159),
 144 (159), 151, 477 (151), 505,
 531 (113), 536

- Long, M. E., 34 (57), 56, 198 (180), 221
- Lopyrin, A. I., 326 (77, 78), 331
- Lorraine, J. A., 90 (103, 104), 100 (104), 109
- Lorenz, N., 90 (7, 8, 9), 102 (7, 8, 9), 103 (7, 8, 9), 106
- Lostroh, A. J., 78 (129, 130), 88 (130), 89 (129), 109
- Lowrey, L. G., 463 (57), 468
- Lowsley, O. S., 43 (58), 57
- Ludwick, T. M., 546 (126, 147), 558 (127), 586 (147), 592, 593
- Ludwig, D. J., 79 (105), 109
- Ludwig, K. S., 21 (80), 25
- Lunn, H. F., 41 (59), 57
- Lutwak-Mann, G., 232 (115), 263, 429 (33, 34, 39), 432, 473 (117), 504
- Lyon, R. A., 89 (106), 109
- Lyons, W. R., 34 (15), 55, 74 (55, 58), 83 (108, 131), 87 (108, 121), 91 (55, 56, 107), 92 (55, 107), 107, 109, 138 (180), 151, 387 (67), 388 (87), 395, 472 (62), 478 (31), 477 (119), 489 (118, 120), 492, 493 (31), 495 (39), 498 (31, 39), 502, 503, 504, 514 (25), 534, 542 (140), 544, 545 (91, 92), 548 (6, 91), 547 (90), 548, 562 (89, 91), 589, 591, 592
- M
- McArthur, J. W., 98 (112), 109
- McCabe, M., 19 (27), 24
- McCann, S. M., 196 (137), 197 (139), 220
- McClean, J. W., 463 (21), 467
- McCullagh, D. R., 69 (167), 110, 125 (243, 244), 128, 152, 153, 154, 197 (198), 221
- McDermott, W. V., 196 (140), 220
- McDonald, A., 228 (77), 262
- McDonald, L. E., 386 (71), 396, 500 (121), 505
- McDonald, M. F., 300 (79), 323 (79), 331
- McDowell, E. C., 463, 464 (58), 468
- McDowell, C. C., 463 (58), 464 (58), 468
- McEntee, K., 224 (167), 225 (89, 167), 226 (89), 230 (167), 231 (167), 234, 238 (167), 247, 249 (89), 256, 258 (89), 262, 263, 264, 390 (65), 395, 520 (100), 536
- McGee, W. R., 275 (61), 288
- McGillard, P. C., 463 (32), 467
- McGraw, R. V., 481 (126), 483 (128), 505
- McKenzie, F. F., 144 (36, 177), 148, 152, 271, 272, 273, 277, 279 (3, 62), 280 (3), 282, 283, 284, 285, 286 (3, 62), 287, 289, 294 (81, 82, 84), 295 (81, 84), 298 (84), 297 (80, 83, 84), 298 (14), 299 (14, 84), 300 (84), 307 (144), 313 (84), 330, 331, 333, 339, 341 (42), 345, 346, 349, 356, 408 (62), 432, 525 (7), 534
- McKeown, T., 47 (97), 57, 189 (46), 217, 517, 536
- McLellan, A. M., 134 (184), 152
- McMahon, B., 517, 536
- McManamny, L. F., 277 (83), 289
- McNaughten, M. J., 481 (157), 483 (157), 508
- McNutt, G. W., 232 (126), 233 (128), 234 (126), 263
- McNutt, S. H., 21 (79), 22 (135, 136), 25, 26, 174 (128), 183, 228 (186), 234 (114), 236 (186), 248 (17, 19, 20), 260, 263, 265, 388 (71), 398, 500 (121), 505
- McPhail, M. K., 139, 152, 468, 477 (122), 505
- McQueen-Williams, M., 553 (94), 591
- McShan, W. H., 252 (38, 39, 189), 253 (166, 189, 192), 255 (166), 261, 264, 265, 310 (93), 332, 470 (196), 507
- Maddock, W. O., 34 (56, 60), 56, 57, 82 (109), 109
- Magnusson, A. M., 136 (54), 149
- Magoun, H. W., 214 (79), 218
- Mahaffey, L. W., 277, 278 (59), 281 (59), 288, 471 (123), 505
- Mai, F., 225 (27), 228 (27), 261
- Maibenco, H. C., 490 (124), 505
- Makepeace, A. W., 138 (161, 162), 151
- Makowski, E. L., 1 (81), 25
- Malan, A. P., 464, 465, 467, 468
- Mall, F. P., 461, 465, 466, 468

AUTHOR INDEX

- Malpress, F H, 139 (40), 149, 242 (22, 23), 253 (68), 260, 262, 491 (51), 503, 540 (32), 542 (32), 564 (52), 566 (50, 51), 577, 579 (53), 590
- Mandl, A M, 19 (82, 83, 84, 85, 86), 25, 213, 214, 220, 231, 263
- Mann, T, 48, 56, 57, 122 (164), 123, 124 (164, 105, 166), 151, 152, 178, 181, 407 (40), 432
- Maqsood, M, 157, 165, 181
- Marberger, E, 1 (87), 25
- March, B E, 109 (15), 180
- Marchetti, A A, 233 (143), 264
- Marden, W G R, 82 (110), 109, 252, 263
- Marder, S N, 532 (102), 536
- Maré, G, 292 (107), 296 (107), 297 (107), 298 (107), 299 (108), 309 (107), 332
- Marion, G B, 228, 253, 257, 263, 586 (20), 589
- Markee, J E, 199, 200, 220, 255, 256 (158), 257 (157, 158), 263, 264, 430, 468
- Mark, J S, 21 (88), 25
- Marker, R E, 481, 483 (120), 505
- Marks, S, 496 (13), 502
- Marrian, G F, 34 (30), 56, 480 (126a), 502, 505
- Marshall, F H A, 119, 152, 187, 190 (146), 191 (145, 146), 205 (145), 220, 292, 295 (86), 299, 331, 364 (69), 372 (46), 373, 374 (09), 376 (69), 382 (68, 69), 384 (69), 385, 387 (69), 389 (14), 390 (14), 394, 395, 396, 473, 494, 505, 513 (61), 515 (103), 535, 536
- Marshall, F R, 294 (87), 331
- Marshall J M, Jr, 475 (128), 505
- Marshall S P, 565 (93), 568, 569 (93), 591
- Marshall W A, 190 (2), 193 (2), 216
- Marsters, R W, 147 (02), 149
- Martin, C E, 200 (128), 219
- Martin, E A, 517 (104), 536
- Martin, L, 136 (168), 152
- Martinez Campos, C, 107, 168 (131), 171, 181, 182, 183
- Martins, T, 104 (111), 109, 115 (109), 123 (109), 152
- Martius, H, 520, 536
- Martynova, N V, 520 (106), 536
- Masina, M H, 123 (136), 151
- Mason, H L, 173 (102), 174 (102), 170 (68), 181, 182
- Mason, K E, 36 (07), 57
- Mason, R G, 79 (13), 106
- Masson, G, 125, 152
- Mathieson, D R, 128 (172), 152, 173 (102), 174 (102), 182
- Matthay, R, 121 (173, 174), 152
- Matthews, L H, 191, 220
- Mauch, A, 270 (60), 272, 273, 276 (26), 288, 518 (107), 519 (107), 536
- Mayer, D T, 34 (40), 56, 164 (20), 180, 337 (55), 350 (55), 356
- Mayer, G S, 517 (117), 536
- Maxwell, E L, 10 (140), 17 (146), 27, 495 (193), 506
- Mazer, G, 128 (175), 152
- Mazer, M, 128 (175), 152
- Mead, S W, 174 (30), 180, 249 (58), 201, 511 (130), 512 (130), 514 (130), 517 (130), 518 (130), 519 (130), 520 (57), 522 (57), 535, 537
- Means, R H, 270 (14), 277 (12, 14), 286 (12), 287
- Mentes, J, 139 (209), 153, 161, 162, 181, 500 (129), 505, 544 (111, 143, 144), 545, 546 (111), 547 (111, 113), 548, 549, 551 (108), 552 (111, 158), 553 (77, 110, 111, 159), 554, 560, 562 (95, 97, 102, 103), 564, 568, 569 (101), 571, 574 (115), 575 (115), 577, 580 (98), 581, 582 (111), 584, 580 (112), 588, 591, 592, 593
- Melampy, R M, 34 (13), 55, 226, 244, 263, 353 (22), 356
- Meller, R E, 124 (179), 152
- Melton, A A, 22 (88), 25, 230 (128), 263, 437, 468, 490 (130), 505
- Meng, C W, 526 (20), 534
- Meranze, D R, 20 (63), 25
- Merkel C G, 126 (190), 152
- Merrick, E H, 140 (180), 152
- Meuleman W L, 581 (150), 593
- Meyer, J, 34 (68), 57

- Meyer, K., 88 (51), 107, 243 (129), 263, 387 (37a), 394
- Meyer, R., 13, 25
- Meyer, R. K., 79 (19, 20, 63), 106, 107, 162, 183, 252 (38, 39, 189), 253 (189), 261, 265, 328 (15), 330, 369 (72), 378 (72), 385 (72), 387 (72), 388 (72), 396, 470 (196), 483 (94), 485 (94), 504, 507, 515 (30), 534
- Mezen, J. F., 193, 219
- Mihaila, M., 304 (88), 315 (88), 331
- Millar, R., 407 (41), 432
- Miller, F. W., 245, 248 (65), 262, 530 (43), 534
- Miller, J. C., 339, 341 (42), 358
- Miller, M. E., 29 (89), 51, 57
- Miller, R. F., 81 (33), 107, 143 (42), 148, 294 (19, 20, 21), 298, 297 (21), 298 (21), 299 (21), 300 (21), 304 (21), 310 (18, 19), 311 (18), 312 (18), 313 (19), 327, 330, 473 (40), 502
- Miller, W. R., 548 (114), 592
- Milligan, J. L., 169 (67), 181
- Mills, L. C., 175 (135), 176 (135), 183
- Milovanov, V. K., 283 (64), 287 (64), 289
- Mirskaya, L. M., 279 (85, 68), 283, 286 (65, 66), 289
- Mitchell, K. C., 558 (18), 589
- Mitchell, W. M., 391 (73), 396
- Mitchison, J. M., 419 (42), 432
- Mixner, J. P., 176 (75, 93), 177 (92, 93), 181, 182, 542 (118), 545, 549 (115), 560 (115), 574 (115), 575 (115), 592
- Moberger, C., 23 (4), 23
- Moeller, A. N., 178 (122), 183, 225 (136), 227, 230 (182), 263, 265, 407 (59), 432
- Moffit, J. C., 343 (52), 356
- Money, W. L., 177, 178, 180, 181, 532 (102), 536
- Montaga, W., 34 (70, 71, 72), 57
- Montané, L., 29 (73, 74), 57
- Moore, C. R., 494 (132), 495, 505
- Moore, L. A., 163 (107), 164, 182
- Moore, N. W., 301, 303, 304 (125), 305, 309 (126), 315 (89, 120), 316 (126), 317 (126), 318 (126), 320 (90), 321, 323 (89, 90, 125), 324, 331, 332
- Moore, R. A., 45, 57
- Moore, W. W., 192, 220, 310 (91, 92, 93, 94), 332, 472 (134), 505
- Moralee, B. E., 490 (82), 504
- Morey, W. L., 146 (3), 147
- Morgan, B. B., 21 (79), 22 (135, 136), 25, 26, 174 (126), 183, 228 (186), 234 (114), 236 (186), 238 (186), 263, 265
- Morgan, C. F., 125 (185), 152
- Morley, T. P., 212 (153), 220
- Moore, C. R., 15 (90, 91), 25, 38 (75), 57, 123 (183), 124, 125 (185, 187), 127 (182), 134 (184), 152, 202, 213 (150), 220
- Morois, M., 174 (27), 180
- Morrell, J. A., 380 (63), 382 (63), 388 (63, 99), 389 (83a), 395, 396
- Morris, C. J. O. R., 140 (32), 148
- Morris, P., 140 (32), 148
- Morrison, F. B., 224, 263
- Morse, A., 78 (85), 108
- Morse, W. L., 218
- Mortara, F., 127 (94), 150
- Moses, L., 472 (135), 505
- Moss, S., 20 (92), 22 (93), 25, 233, 236, 238 (133), 244 (169), 263, 264, 472 (138), 500 (138), 505
- Moss, W. P., 365, 396
- Mossman, H. W., 434 (64), 435, 438, 439, 440, 442 (64, 65), 443, 444 (64), 445, 446 (65), 448, 449, 450, 451, 452, 453 (64), 459 (64), 461 (65), 468
- Motohashi, K., 258 (137), 263
- Moulder, P. V., 34 (46), 56
- Moustgaard, J., 338 (32), 352 (32), 356
- Mu, J. W., 566 (58), 590
- Muller, H. G., 21 (94), 25
- Muller, M. E., 531 (108), 536
- Mullins, M. C., 86 (113), 109
- Mullick, D. N. B., 584 (117), 592
- Mulligan, R. M., 304 (75), 371 (75), 373 (75), 374 (75), 376, 378 (75), 383, 390 (70), 396
- Munson, P. L., 555, 592
- Munson, T. O., 72 (27), 106

Murphree, R L, 326 (93), 332
 Murphy, H S, 241 (134), 263
 Murray, G H, 390, 396
 Murrar, J A, 463 (66), 468

N

Nader, D N, 125 (218), 126 (218), 153
 Nahm, L K, 297 (80), 331
 Nalbandov, A V, 81 (114, 115), 109, 192, 220, 252 (38), 261, 295 (149), 305 (67), 307 (67), 310 (91, 92), 331, 332, 333, 336 (30), 342, 348, 356, 472 (134), 475 (137), 476 (137), 501 (197), 505, 507
 Nass, C A G, 100 (162), 110
 Neher, G M, 84 (175), 110, 118, 152, 154, 308, 332, 355 (69), 357, 474 (138), 486 (138), 491, 501 (138), 505, 529 (109, 167), 532 (169), 536, 538
 Nellor, J E, 251, 263, 472 (139), 505, 568 (119), 574, 592
 Nelson, J W, 96 (22), 106
 Nelson, K R, 387 (102), 396
 Nelson, M M, 87 (116, 117, 118, 119, 120, 121, 151, 174), 90 (40), 104 (40), 107, 109, 110, 516, 536
 Nelson, W O, 1 (87, 96), 2 (95), 25, 34 (56, 60), 56, 57, 82 (109), 109, 126 (190), 139 (191), 152, 478 (140), 505, 529 (111), 536, 554 (122, 123), 559, 560, 592
 Nelson, W W, 2 (97), 25
 Newberry, W E, 364 (78), 374, 380, 396
 Newton, B L, 175 (135), 170 (135), 183
 Newton, W H, 140, 147, 150, 471 (143), 474 (141), 491, 505, 531 (156), 537, 547 (124), 592
 Nibler, C W, 253 (170), 265, 193 (144), 505
 Nicholas, J S, 424 (41), 429 (43), 432
 Nichols, C W, 2 (98), 25, 490 (110, 200), 504, 507
 Nichols, R E, 396 (71), 396, 500 (121), 505
 Nicmann-Sørensen, A, 77 (31), 107
 Nilsson, L, 136 (51), 149

Nilsson, O, 21 (10), 23
 Nishikawa, M, 258 (137), 263
 Nishikawa, Y, 34 (77, 78), 57, 270, 277 (68, 69), 289
 Noble, R L, 126 (192), 152
 Noda, Y, 76 (123), 86 (123), 109
 Nordstrom, A, 39 (19), 56
 Norton, H W, 192 (154), 220, 295 (149), 305 (67), 307 (67), 310 (94), 331, 332, 333, 336 (30), 356, 501 (197), 507
 Novikoff, M, 169 (44), 181
 Nowell, N W, 190 (155), 220
 Nowinski, W W, 402 (26), 431
 Nussbaum, N, 115 (193), 152

O

Ober, W B, 8 (100), 12, 26
 O'Donnell, V J, 114 (120, 121), 150
 Odor, D L, 299 (97), 332
 Oehler, I E, 3 (101), 26
 Ohno, F, 258 (137), 263
 Okamoto, T, 373, 396
 Okey, R, 343 (9), 355
 Olds, D, 225, 228, 229, 230 (144), 243, 263, 264
 O'Leary, J L, 2 (99), 21 (99), 25
 Olvecrona, H, 210 (123), 216, 219, 220
 Oloufa, M M, 169, 181
 Olsen, A G, 140 (220), 153
 Olsen, N H, 338 (32), 352 (32), 356
 O'Mary, C C, 311 (98), 320 (98), 328 (98), 332
 Onuma, H, 34 (78), 57
 Orsós, F, 23 (102), 26
 Ortavant, R, 291 (23), 310 (136), 313 (23), 325 (23), 320 (24, 137), 329 (23), 330, 333
 Osborn, S B, 137 (22), 148
 Oshima, M, 500 (64), 590
 Ostlund, H L, 33 (4), 55
 Otsuka, H, 76 (123), 86 (123), 109
 Ottaway, C W, 189, 502

P

Paduana, A L, 320 (159, 160, 161), 333
 Page, F J A, 79 (125), 104 (121, 125), 109
 Page, F, 459 (120), 504

- Pállson, H., 328 (99), 332
 Pan, S. C., 472 (29), 494 (29), 495 (29), 502
 P'an, S. Y., 78 (161), 110
 Panattoni, M., 480 (126a), 505
 Papadatos, C., 116 (41), 148
 Papadopoulos, J. C., 294, 332
 Papanicolaou, G. N., 131, 137 (195), 152, 153, 233 (143), 264, 379, 396
 Papkoff, H., 78 (126), 109
 Pappalardo, C., 23 (103), 26
 Paredis, F., 227, 265
 Park, W. W., 2 (104), 26
 Parker, G. H., 299 (101), 332
 Parkes, A. S., 20 (105), 26, 34 (30), 56, 115 (198), 117 (79), 121 (96), 126 (77, 78, 141), 133, 134 (76), 144, 145 (124), 149, 151, 152, 188 (121), 191 (157), 205 (157), 219, 220, 310 (58), 311 (58), 312 (58), 328 (58), 331, 387 (80), 396, 472 (148), 492 (145), 505
 Parkins, W. M., 388 (99), 396
 Parsons, U., 48 (86), 57
 Paschalis, K. E., 124 (228), 153, 482 (149), 484 (149), 505
 Patten, B. M., 382 (81), 398, 483, 488
 Patterson, H. D., 19 (86), 25, 231 (118), 263
 Patzelt, V., 20 (108, 107, 108), 26
 Paulsen, G. A., 34 (58), 56, 82 (109), 109
 Payne, F., 171, 181
 Payne, R. W., 132, 152
 Pearlman, W. H., 481 (147, 148), 482 (149), 484 (149), 485 (147), 487 (147), 505
 Pearse, A. G. E., 475 (150), 505
 Pearson, K., 364 (82), 372 (82), 384 (82), 393, 396
 Pearson, M., 364 (82), 372 (82), 384 (82), 393, 396
 Pearson, O. P., 514 (112), 536
 Pedersen, K. O., 76 (100), 88 (99), 108
 Pearson, P. B., 488 (28), 502
 Peel, W. R., 515 (103), 536
 Pele, T. L., 192, 221
 Pencharz, B. I., 68 (57), 74 (57), 83 (127), 107, 109, 132, 152, 477 (151), 505, 531 (113), 536
 Perkins, J. R., 230, 264
 Perl, E., 474 (152), 505
 Perlmutter, J., 193 (190), 213 (191), 221
 Perry, J. S., 524, 525 (114), 536
 Petersen, W. E., 163 (101), 184 (101), 185 (80), 181, 182, 250 (168), 254 (100), 262, 264, 548, 553, 555 (42), 558, 582 (14, 36, 83), 583 (14), 584 (14), 588 (147), 589, 590, 591, 592, 593
 Peterson, R. R., 185 (136, 137), 183
 Petropavlovsky, V. V., 279 (65), 286 (85), 289
 Pfeiffer, C. A., 202, 220, 472 (153), 505
 Pfiffner, J. J., 139 (191), 152, 387 (83), 396, 529 (111), 536
 Philipp, E., 89 (128), 109, 493 (154), 505
 Phillips, D. S. M., 533 (12), 534
 Phillips, K., 555 (37), 590
 Phillips, R. W., 34 (80), 40 (79), 57, 294 (81, 82), 295 (81), 297 (132), 328, 329, 331, 332, 333, 337, 356
 Phillips, W. A., 138 (198), 152
 Piaux, G., 530 (135), 537
 Pickard, J. N., 518 (115), 520 (115), 536
 Pickford, M., 218 (1), 218
 Pilka, H. J., 38 (49), 56
 Pincus, G., 113 (201), 115 (200), 118, 131 (199), 152, 427 (45, 46, 47), 429 (28, 48), 431, 432, 492 (78, 155), 493 (155), 494 (78), 503, 505, 529 (136), 537
 Pinsky, P., 189, 180
 Pipes, G. W., 158 (81, 82, 83), 159 (85), 160 (84), 170 (83, 85a), 182
 Plas, J., 270 (1), 277 (1), 287
 Plate, W. P., 20 (109), 28
 Plotz, E. J., 478, 479, 496 (52), 497 (53), 503
 Pohley, F. M., 92 (155), 93 (155), 110
 Polge, G., 178 (69), 181, 407 (40), 432
 Politzer, C., 13 (110), 26
 Polley, H. F., 173 (102), 174 (102), 182
 Pollock, W. F., 34 (81), 57
 Polovtzeva, V. V., 304 (104), 329 (105), 332
 Pomerantz, L., 86 (113), 109

- Pomeroy, B. S., 163 (101), 164 (101),
 165 (80), 181, 182, 250 (168), 264,
 546 (126), 592
 Pomeroy, R. W., 346, 356, 471 (156),
 505
 Pomeroy, W. B., 206 (128), 219
 Pope, A. L., 298 (39), 311 (98), 326
 (98), 328 (98), 330, 332, 501 (66),
 503
 Pope, G. S., 481 (157), 483 (157), 506,
 586 (128), 592
 Popenoc, E. A., 78 (45), 107
 Potter, C. D., 120 (202), 152
 Potts, C. G., 294 (87), 331
 Potvin, R., 131 (45), 149
 Pou, J. W., 225, 264
 Poulton, B. R., 554 (129), 592
 Pounden, W. D., 555 (71), 591
 Power, M. H., 173 (102), 174 (102),
 182
 Pozo Lora, R., 518 (116), 536
 Pratt, J. M., 169 (45), 181
 Preedy, J. R. K., 117, 152
 Prem, K. A., 1 (81), 25
 Premachandra, B. N., 158 (82, 83), 159
 (85), 160, 170 (83, 85a), 182
 Preuss, F., 21 (111), 26
 Price, D., 16 (112), 26, 123 (204), 124,
 125 (187), 126 (205), 152, 153,
 202, 220
 Prichard, M. M. L., 195 (55, 56), 217
 Primrose, T., 487 (190), 506
 Fund, E. R., 387 (44), 389 (44), 395
- Q
- Quick, W. J., 38 (75), 57
 Quimby, E. H., 496 (178), 506
 Quin, J. L., 299 (106), 313 (106), 332
 Quinlan, J., 270, 289, 292 (107), 296
 (107, 109), 298 (107), 299 (108),
 309 (107), 332, 517 (117), 536
- R
- Raacke, I. D., 70 (129, 130), 88 (130),
 89 (129), 109
 Radford, H. M., 292 (148), 301 (112),
 332, 333
 Raeside, J. L., 118, 153, 251, 261, 300
 (70), 323 (70), 331
 Ragdale, A. C., 584 (130), 592
 Rakes, J. M., 226 (127), 244 (127), 263
 Rakoff, A. E., 96 (70), 108, 482 (149),
 484 (149), 505
 Ralston, N. P., 584 (130), 592
 Randall, S. P., 497 (191), 506
 Rankin, R. M., 496 (158), 506
 Ranold, A. E., 218
 Ranson, R. M., 187, 216
 Ranson, S. W., 197 (35, 62), 198 (62),
 208, 214 (63, 79), 217, 218, 531
 (36), 534
 Raps, G., 362 (84), 396
 Rasbech, N. O., 228 (10), 260, 520
 (118), 525 (119), 536
 Rasmussen, A. T., 193 (159), 210 (159),
 220
 Ravera, M., 116 (207), 153
 Ray, E. W., 83 (131), 109, 546 (6), 589
 Raynaud, A., 16 (113, 114, 115), 17
 (113, 115), 26, 134 (208), 153,
 495 (159), 506
 Rayner, B., 165 (137), 183
 Reardon, T. F., 321 (127), 332
 Reece, R. P., 2 (35), 22 (35), 24, 164,
 182, 230 (147), 264, 491, 508, 544
 (134), 548, 549 (131), 554 (129),
 555 (132), 566 (131, 132), 578
 (133), 592
 Regan, W. M., 520 (57), 522 (57), 535
 Reichert, F. L., 387 (37a, 67, 85), 388
 (67), 394, 395, 396, 547 (90), 591
 Reid, C. H., 497 (161), 506
 Reid, D. E., 532 (1), 533
 Rein, C., 527 (120), 536
 Reincke, E. P., 139 (209), 153, 158 (52,
 88, 123), 160, 101, 102, 104, 105,
 167 (111), 168 (112, 131), 171
 (131), 180, 181, 182, 183, 250
 (148), 264, 544 (101), 555, 568
 (100, 110), 569 (101), 571 (100,
 130), 574, 577 (100, 130), 581, 582
 (14, 135), 583 (14), 584 (14, 100,
 117), 580 (09), 589, 591, 592, 593
 Reynolds, S. R. M., 2 (110), 21 (110),
 26, 131 (210), 140 (211), 153, 258
 (149), 264, 471 (102), 490 (162),
 506, 510 (122), 523, 525 (122),
 527, 529 (121), 530 (2, 122), 533,
 536
 Rice, V. A., 267, 286 (72), 299

- Richardson, K. C., 139 (46), 149, 491
(51), 503, 540 (32, 138), 542 (32),
547 (124), 558, 590, 592
- Riches, J. H., 294 (111), 332
- Richter, F., 304 (112), 332
- Rickard, C. G., 390 (65), 395
- Riddle, O., 547 (139), 592
- Riddoch, G., 209 (45), 217
- Rife, D. C., 511 (17), 512 (17, 123),
513 (54), 514 (17), 516 (17), 517
(17), 518 (17), 534, 535, 536
- Riley, C. M., 102 (172), 110, 190 (160),
220
- Rimington, C., 76 (132), 88 (132), 109
- Ripstein, M. P., 497 (181), 506
- Rioch, D. McK., 198 (137), 208, 218,
220
- Riser, W. H., 396, 398
- Rittau, M., 304 (112), 332
- Roark, D. B., 225 (150), 234 (150), 236
(150), 238 (150), 239, 242, 243,
284
- Roberts, S., 254, 284, 473 (181), 482
(180), 491 (180), 508
- Roberts, S. J., 2 (117), 28, 225 (7), 229
(151), 234 (7), 238 (7), 238 (7),
241 (82), 244 (5, 7), 245 (5), 248
(5, 82), 247 (5), 250, 253 (5), 260,
262, 264, 303 (59), 331, 366 (87),
382 (87), 388 (87), 389, 390 (87),
396, 520 (100), 523 (124), 524, 525
(124), 528 (124), 536
- Robertson, C. L., 338 (45), 339, 350,
358
- Robertson, P. A., 531 (65, 66), 535
- Robertson, W. C., 176 (92), 177, 182
- Robey, M., 530 (135), 537
- Robinson, B., 198 (173), 208, 220
- Robinson, D., 496 (32), 502
- Robinson, G. E., Jr., 342, 348, 356
- Robinson, T. J., 81 (133), 134), 109,
138 (212), 153, 292 (115), 294,
295 (115), 297 (113), 299 (114),
300 (125), 301, 303, 304 (125),
305, 309 (118, 121, 126), 310
(115), 311 (113, 117, 122), 312,
313, 314, 315, 316, 317, 318, 319,
320 (90), 321, 322 (124), 323 (89,
90, 125), 324, 325 (115, 116, 117,
119, 121, 124), 326 (114, 122), 327,
328 (122, 123), 331, 332, 489
(163), 506
- Robson, J. M., 114 (216, 217), 126 (214,
215), 137 (213), 153, 214 (181,
162), 220, 385 (88), 387 (88), 390
(88), 396, 529 (126, 127), 530
(125, 128), 531 (13), 534, 537
- Rocha, A., 115 (169), 122 (169), 123
(189), 152
- Roepeke, M. H., 204 (118), 219
- Rogers, A., 212 (185), 220
- Rogoff, J. M., 387 (89, 90), 396
- Rollandi-Racci, V., 116 (207), 153
- Rollins, W. C., 270 (49, 73), 288, 289,
511 (130), 512, 514, 515 (75), 517
(130), 518, 519 (130), 535, 537
- Romanoff, E. B., 492 (78), 494 (78),
503
- Ronaldson, J. W., 308, 309, 330
- Rorik, H. H., 457 (67), 465, 488
- Rosa, P., 5 (118), 9 (118), 26
- Rosahn, P. D., 518 (131), 537
- Rosenberg, E., 198 (137), 220
- Rosenburg, T., 78 (25), 82 (25), 106
- Ross, J. F., 215 (188), 221
- Ross, M. A., 123 (152), 151
- Rothen, A., 78 (23), 106
- Rothschild, Lord, 400 (49), 409 (49),
432
- Rottenstein, K., 228, 264
- Roussy, C., 192, 217
- Routh, A., 527 (132), 537
- Roux, L. L., 294 (128), 295 (128), 299
(108), 332, 333, 517 (117), 536
- Rowan, W., 186 (164), 187, 189, 220
- Rowland, S. J., 581 (44), 590
- Rowlands, I. W., 76 (132), 88 (132),
109, 362 (47), 364, 374, 379 (47),
380 (47), 382 (47), 384 (47), 389
(47, 47b), 391, 392, 393, 395, 396,
455, 467, 468, 488, 497 (2), 498
(55), 499 (2), 501, 503
- Rowson, L. E. A., 81 (135), 109, 176,
181, 182, 246 (111, 154, 155), 251
(32, 153), 252, 253, 261, 263, 264,
407 (40, 50), 432, 470 (164), 506
- Boy, A., 81 (64), 107
- Rozin, S., 94 (14), 96 (14), 106
- Rubenstone, A., 20 (63), 25

- Rubin, I C, 21 (78), 25
 Rubinstein, H S, 125 (218), 126, 153
 Rudolph, G G, 124 (166), 152
 Rumbolz, W L, 22 (119), 26
 Runner, M H, 476, 504
 Rupel, W, 230 (36), 261
 Ruppert, H L, 551 (162), 568 (162),
 571 (162), 577 (162, 163), 586
 (163), 593
 Rush, H P, 212 (165), 220
 Ruzicka, L, 128, 153
- S
- Saez, F A, 402 (26), 431
 Saki, S, 369 (72), 378 (72), 385 (72),
 387 (72), 388 (72), 396
 Santo, S, 202 (185, 186, 187), 221, 258
 (161, 162, 163), 264
 Salhanick, H A, 79 (136), 109, 140
 (220), 147 (142), 151, 153, 532
 (168), 538
 Salisbury, G W, 229 (160, 181), 264,
 265, 471 (186), 506, 581 (5), 589
 Salisbury, S M, 511 (17), 512 (17), 514
 (17), 516 (17), 517 (17), 518
 (17), 534
 Salmon, U J, 126 (221), 127 (222), 153
 Salter, W T, 478 (165), 506
 Salzman, A A, 279 (66), 283, 286 (66),
 289
 Sammartino, R, 143 (0), 148, 364 (11),
 370 (11), 371, 372, 373 (11), 374
 (11), 376, 378 (11), 382, 383, 380,
 387, 388, 389 (11), 391 (11), 394
 Samuels, L T, 124 (166), 152
 Sanders, D A, 565 (93), 568 (93), 569
 (93), 591
 Sandritter, W, 22 (56), 24
 Satoh, S, 268, 289
 Santolucito, J A, 209 (48), 217, 323,
 333
 Sauerbruch, F, 528, 537
 Saul, G D, 201 (166), 220
 Saunders, H L, Jr, 176 (75), 181
 Saunders, J R de C, 189 (117), 219
 Saunders, F. J, 182 (41), 183 (41), 188
 (41), 502
 Saunders, P J, 69 107
 Sauramo H, 20 (120), 26
 Savage, J F, 169 (95), 182
 Sawyer, C H, 2 (121), 26, 196 (173),
 199, 200, 201, 208, 216, 217, 218,
 220, 255 (122, 124), 256 257 (157,
 158), 263, 264
 Sayers, G, 195 (43), 207 (176), 217,
 221
 Sayers, M A, 207 (176), 221
 Saxton, J A, 221
 Scales, J W, 271 (13), 286 (13), 287
 Scammon, R E, 12 (122), 26, 466, 468
 Schaffhausen, D D, 501 (166), 506, 581
 (82), 591
 Scharf, G, 542 (140), 592
 Scharrer, B, 257, 264, 479 (167), 506
 Scharrer, E, 257, 260, 264, 479 (167),
 506
 Schauder, W, 444 (70), 455, 468
 Schuld, H O, 529 (127), 537
 Schunkel, P G, 294 (130, 131), 333
 Schlotthauer, C F, 47, 57
 Schmidt, K, 338, 339, 340, 347 (47),
 349 (47), 356
 Schneider, H, 463 (71), 464 (71), 468
 Schneider, W G, 89 (137), 109
 Schnetzler, E E, 171, 180
 Schoen, H C, 213 (32a), 217
 Schott, R G, 297 (132), 299 (132), 329
 (103), 332, 333
 Schotterer, A, 301 (92), 370, 396
 Schreiner, L, 210, 221
 Schreiner, L H, 196 (137), 220
 Schultze, A B, 158 (96), 170 (96), 171,
 182
 Schultze, O, 402 (72), 405, 468
 Schweizer, M, 196 (186), 221
 Scorgie, N J, 389, 396
 Scott Blair, G W, 496 (71), 503
 Scott-Watson, H M, 566 (54), 590
 Scott, W W, 34 (16), 55
 Scow, R O, 196 (161, 181), 219, 221
 Scully, R E, 72 (27), 106
 Sears, R M, 520 (100), 536
 Seath, D M, 225, 228, 229, 230 (141),
 263
 Seay, P, 193 (190), 213 (161), 221
 Seckinger, D L, 346 356
 Seegar, G F, 89 (71), 109, 493 (73),
 503
 Seeman A, 117 (234), 153
 Segaloff, A, 70 (152), 110

- Seidel, F., 424 (51, 52), 432
 Seidenstein, H. R., 581 (5), 589
 Seifter, J., 174, 182
 Self, H. L., 337, 338, 350, 351 (49, 71), 356, 357
 Selye, H., 40 (88), 57, 116 (223), 117 (9), 137 (224), 148, 153, 190, 195 (85), 218, 221, 475, 506, 545 (142), 555, 592
 Semans, J. H., 204, 221
 Sethre, A. E., 496 (169), 506
 Severinghaus, A. E., 122 (225), 153
 Sewell, O. K., 307 (8, 9), 329
 Sgouris, J. T., 544 (143, 144), 582 (102, 103), 584 (143, 144), 591, 592, 593
 Shaffner, C. S., 167, 169 (38, 98), 171, 181, 182
 Shannon, E. P., 229 (160), 264
 Shapiro, H., 427 (47), 432
 Shapiro, H. A., 125 (226, 227), 153
 Shaver, S. L., 38 (67), 57
 Shaw, H. E. B., 292 (89), 294 (69), 331
 Shaw, J. C., 579, 580, 581 (146), 582, 589, 593
 Shay, H., 124 (228), 153
 Shealy, C. N., 192, 221
 Shedlovsky, T., 78 (23, 161), 106, 110
 Sheets, E. W., 270 (14), 277 (14), 287
 Shelesnyak, M. C., 144 (229), 153
 Shibusawa, K., 202 (185, 186, 187), 221, 258, 264
 Shier, F. L., 132 (11), 148, 294 (138, 139), 333
 Shipley, E. G., 207 (40), 217
 Shipley, R. A., 113 (63), 114, 116, 120, 121, 124, 129, 149
 Shipounoff, G. C., 122 (242), 153
 Shippin, O. F., 531 (108), 536
 Short, R. V., 479 (12), 492, 502, 506, 529, 537
 Shultz, F. T., 385 (8), 394
 Shumway, W., 462 (73), 468
 Siebold, H. R., 385 (113), 397
 Sikes, D., 355 (69), 357, 532 (169), 538
 Silber, R. H., 173 (134), 183
 Silva, A., 115 (170), 152
 Silfverskiöld, W., 210 (123), 219
 Silvestrini, D. A., 169 (15), 180
 Simeone, F. A., 215 (188), 221
 Simmonet, H., 147 (25), 148, 530 (135), 537
 Simmons, V. L., 328 (34), 329 (103), 330, 332, 574 (38), 590
 Simon, J., 246 (17, 19, 20, 21), 260
 Simpson, E. C., 168, 180
 Simpson, M. E., 88 (57), 89 (139, 141, 142), 71 (139, 141, 142), 72 (58), 74 (55, 56, 57, 69, 142), 78 (101), 77 (52), 78 (165, 166), 83 (21, 163, 168), 85, 87 (174), 88 (51, 53), 89 (106), 90 (38, 52, 54, 164), 91 (55, 56, 58, 102), 92 (52, 55, 58, 140, 173), 93 (58, 140), 99 (52), 101 (54), 103 (52, 58, 68, 138, 143), 104 (38, 143), 105 (68), 106, 107, 108, 109, 110, 199 (195), 221, 387 (37a), 394, 472 (62), 476 (171), 503, 506, 530 (51), 535
 Simpson, S., 164, 182
 Sinclair, D. P., 328 (142), 333
 Singerman, L. S., 348, 358
 Sisson, S., 2 (123), 26, 29 (87), 41 (87), 42 (87), 57, 229 (164), 264, 370 (94), 398
 Sizemore, J. R., 189 (67), 181
 Skjerven, O., 22 (124), 28, 236, 237 (185), 238, 242 (71), 262, 204
 Slaughter, I. S., 558, 593
 Slocum, D., 212 (165), 220
 Slocumb, C. H., 173 (102), 174 (102), 182
 Sloper, J. C., 257, 260
 Smiley, E. S., 581 (99), 588 (99), 591
 Smith, A. U., 429 (53), 432
 Smith, F., 392 (95), 396
 Smith, G. M., 542 (61), 590
 Smith, H. A., 390 (96), 396
 Smith, J. D., 209 (48), 217
 Smith, M. G., 529 (137), 537
 Smith, O. W., 529 (138), 537
 Smith, P. E., 60 (144, 146, 147), 66, 79 (148), 83 (149), 110, 131 (230), 153, 213 (189), 221, 470 (172), 476, 477 (174), 478 (175), 493 (175), 506, 530 (138, 140), 531 (139), 537
 Smith, V. R., 226 (4, 119), 227 (4), 253, 255 (166), 257, 260, 263, 264, 478 (176, 179), 506

- Smith, W. W., 350 (50), 356
 Smithcors, J. F., 545 (81), 591
 Smythe, R. H., 524 (141), 537
 Snelling, F. G., 146, 153
 Snyder, F. F., 345, 356, 529 (142), 537
 Snyder, L., 512 (123), 536
 Soliman, F. A., 101 (89), 162, 182, 250
 (148), 264, 307 (41), 330
 Sommerville, I. F., 118, 140 (232), 153
 Sorensen, A. M., 224, 225 (167), 230
 (167), 231 (167), 234 (167), 230
 (167), 264
 Sorterup, E., 520 (143), 537
 Spalding, J. F., 343, 356
 Spector, W. S., 472 (177), 506
 Speert, H., 496 (178), 506
 Spencer, E., 517, 537
 Spiegelberg, O., 510, 537
 Spielman, A., 165 (80), 181, 546, 586
 (147), 593
 Spielman, A. A., 103 (101), 164, 182,
 250 (108), 264
 Spiritos, B. M., 192 (32), 217
 Spürri, H., 351 (53, 51), 356
 Sprague, R. G., 173, 174, 182
 Spreull, J. S. A., 393 (97), 396
 Squire, P. G., 70 (150), 110
 Squiers, C. D., 337, 350, 356
 Srebnik, H. H., 87 (151), 110
 Stoffe, A., 518 (140), 537
 Stange, H. H., 17 (125), 20 (125, 126),
 21 (126, 127), 26, 89 (41), 107
 Stanley, A. J., 506 (164), 593
 Starke, N. C., 178 (163), 182, 299
 Stormont, C., 520 (89, 148), 521 (89,
 148), 531 (89), 536, 537
 Stotsenberg, J. M., 464 (74), 468
 Stott, G. H., 478 (170, 179), 506
 Strahl, H., 459 (75), 468
 Streeter, G. L., 2, 11, 25, 26, 424 (32),
 432, 446 (45, 46), 462, 467, 468
 Straker, P., 547, 593
 Strong, L. C., 542 (61), 590
 Struve, J., 339, 357
 Stumme, E., 475, 503
 Sturgis, S. H., 80 (150), 90 (157), 164
 (159), 110
 Sturkie, P. D., 168, 180
 Sudan, A. C., 555 (37), 590
 Sugge, T., 270 (67), 289
 Sulman, F. G., 72 (87), 77 (178), 94
 (14), 90 (14), 101 (178), 106,
 108, 110, 130 (231), 153
 Swezy, O., 369 (38), 370 (38), 373, 383
 (38), 395
 Swingle, W. W., 193 (190), 213, 221,
 387 (83), 388 (99), 396
 Swinyard, C. A., 195 (43), 217
 Sykes, J. F., 20 (02), 22 (03), 25, 225
 (145), 233, 236 (133), 238 (133),
 244 (169), 254, 260, 263, 264, 292
 (134), 333, 472 (130), 500 (130),
 505, 512 (151), 574, 591 (150,
 167), 580, 590, 593
 Szengogothai, J., 108 (102), 221
 Szego, G. M., 254, 264, 473 (161), 482
 (180), 491 (180), 506

- Templeton, H. J., 138 (160), 151
 Terrill, C. E., 144 (177), 152, 294 (84),
 295 (84), 296 (84), 297 (80, 83,
 84), 299 (84), 300 (84), 313 (84),
 331, 511 (150), 513 (150), 515
 (150), 517 (150), 519, 537
 Tgetgel, B., 179, 182
 Thibault, C., 294 (23), 310 (136), 313
 (23), 325 (23), 326 (24, 74, 135),
 329 (23), 330, 331, 333
 Thimann, K. V., 113 (201), 152
 Thomas, J. W., 163 (107), 164, 182, 582,
 583 (153), 593
 Thomson, A. M., 515 (151), 537
 Thomson, A. P. D., 190 (2), 193 (2),
 216
 Thomson, D. L., 475 (188), 492 (42),
 502, 506
 Thomson, W., 515 (151), 537
 Thorn, D. W., 387 (102), 396
 Thorn, C. W., 218, 387 (101, 102), 398
 Thorpe, F. J., 500 (129), 505
 Tillotson, C., 115, 151
 Tindal, J. S., 554 (33), 590
 Titus, H. W., 188, 182
 Tobey, E. R., 555 (65), 590
 Tobin, C. E., 478 (140), 505
 Tokuyama, I., 34 (58), 58, 82 (109),
 109
 Tolkdorf, S., 72 (58), 91 (58), 92 (58),
 93 (58), 103 (58), 107
 Tomizawa, K. T., 202 (188), 221, 258
 (182, 183), 284
 Toothill, M. C., 37 (88), 57
 Torda, C., 526 (152), 537
 Tornblom, N., 118, 153
 Torpin, R., 127 (94), 150, 524 (185),
 526 (163), 531 (164), 537, 538
 Torrey, T. W., 4 (130), 26
 Torstveit, O., 175, 181
 Touchberry, R. W., 220, 264
 Townsend, B. F., 200 (172), 220
 Traut, H. F., 233 (143), 264
 Trautmann, A., 2 (131), 26, 29 (89), 32
 (89), 38 (89), 42, 44, 48 (89),
 51 (89), 57, 369 (103), 370, 371
 (103), 383 (103), 396
 Trentin, J. J., 545 (154), 554 (104, 152),
 555 (152), 591, 593
 Trimberger, C. W., 225, 228, 227, 228
 (171, 175), 229 (173, 175), 233,
 251, 253, 255, 262, 264, 265, 472
 (80), 503
 Troland, C. E., 212 (193), 221
 Trum, B. F., 273, 274, 275, 276, 279
 (75), 280 (75), 288 (75), 289
 Tsukaguchi, R., 373, 396
 Turner, C. D., 2 (132), 26
 Turner, C. E., 383, 388 (105), 396
 Turner, C. W., 118, 153, 158 (12, 13,
 14, 81, 82, 83, 96), 159 (85), 160,
 164, 167, 188, 169, 170 (14, 83,
 85a, 96) 171, 172, 180, 181, 182,
 183, 230 (147), 253, 254, 259 (76),
 262, 264, 265, 483 (144), 491
 (184), 505, 506, 540 (158), 542,
 544 (111), 545, 546 (111, 114),
 547 (87, 111, 113), 548, 549, 551
 (41, 108, 162), 552 (111, 158), 553
 (110, 111, 159), 554, 555, 558, 559
 (165), 560, 564, 588 (85, 86, 131,
 132, 157), 568, 569 (89), 571, 574
 (115), 575 (115), 577, 578, 582
 (67, 111), 584, 588, 589, 590, 591,
 592, 593
 Turner, W., 448 (80), 468
 Turpeinen, K., 74 (58), 82 (58), 91
 (56), 107
 Tuttle, L. L. D., 175 (135), 176 (135),
 183
 Tyler, A., 410 (15, 55), 427 (54), 431,
 432
 Tyler, C. M., 530 (37), 531, 534
 Tyler, W. J., 224 (95), 228 (34), 229
 (34), 261, 262, 528 (22), 534
 Tyler, W. S., 38 (51), 48 (51), 47 (51),
 48 (51), 56
- U
- Ulberg, L. C., 246 (19), 251 (177, 178,
 179), 252 (18), 260, 265, 353, 354
 (5), 355, 357
 Umbaugh, R. E., 82 (160), 110, 252
 (180), 265, 470 (185), 506
 Underwood, E. J., 132 (11), 148, 294
 (133, 139), 333
 Ungar, F., 480 (57), 484 (57), 485
 (57), 503

- Uppenborn, W, 268 (76), 270 (76),
289, 518 (153), 537
Uren, A W, 341 (2), 353 (2), 355, 526
(154), 537
Usei, K, 192 (135), 219
- V
- Valdes Dapena, M A, 2 (133), 26
Valerani, L, 512 (14), 517 (14), 534
Van Demark, N. L., 178, 179, 181, 183,
225 (130), 227, 229 (160, 181),
230 (182), 243, 245, 246, 247,
257, 262, 263, 264, 265, 407 (56,
57, 58, 59), 432, 471 (186), 506
Vandeplasseche, M, 227, 265
van der Horst, C J, 142 (131), 151
van der Lee, S, 193 (194), 221
Van der Stricht, R, 418 (60), 432
van der Wath, J G, 299 (106), 313
(106), 332
van der Werff ten Bosch, J J, 188 (67),
190, 201 (68), 213 (68a), 218
Van Dyke, D C, 199 (195), 221
Van Dyke, H B, 76 (23, 161), 93 (74),
98 (74), 106, 108, 110, 131 (64),
149, 197 (98), 219, 475, 506
van Gilse, H A, 100 (162), 110
Van Herden, J S, 270 (77), 289
van Oort, G J, 117 (28), 121 (194),
148, 152
Van Rensburg, S W J, 276 (71, 77),
289
van S Smith, G, 529 (130), 537
van Wageningen, G, 12 (148), 14, 15
(148), 27, 37 (96, 91), 57, 78 (165,
166), 83 (165, 166), 87 (163), 90
(164), 103 (143), 104 (143), 110,
476 (171), 503, 506, 531 (156),
537
Varadi, K, 23 (72), 25
Varangot, J, 117, 153
Vars, H M, 357 (83), 396
Vasquez-Lopez, E, 193 221
Vatna, S, 124 (239), 153
Veiga J S, 512 (82, 83), 535
Velardo J T, 173, 174, 181, 532 (78),
537
Venning F M H, 75 (16), 106, 474
(189), 486 (189), 487 (190), 492
(189), 497 (161, 191), 502 506
- Venzke, W G, 229 (37), 261, 525
(24), 534
Vilas, E, 13 (134), 26
Village, P A, 401 (65), 432
Villeg, C A, 526 (155), 537
Vogt, M, 193, 221
von der Heide, A, 528, 535
Von Lam, L, 118, 151
von Oettingen, B, 518, 537
Vosburgh, G J, 526 (49), 535
Voss, H E, 124 (240), 153
- W
- Wade, N J, 133 (241), 153
Wainman, P, 122 (242), 153
Waldorf, D P, 337 (27), 356
Walker, C W, 478 (176), 506
Walker, R M, 516 (18), 534
Walker, S M, 566 (164), 593
Wallace, L R, 326 (140, 141, 142), 333
Wallach, D P, 158 (123), 183
Walsh, E L, 69 (167), 110, 125 (243,
244), 153, 154, 197 (198), 221
Walter, F X, 189 (24), 217
Walton, A, 142 (245), 154, 366 (109),
397, 400 (61), 432
Wang, Y K, 526 (20), 534
Warbinton, V, 164, 180, 297 (80), 298
(143), 307 (144), 331, 333, 408
(02), 432
Ward, D H, 158, 183
Warner, D E, 496 (13), 502
Warner, E D, 102, 183
Warnick, A C, 228 (185), 229, 265,
337, 356 (62), 352 (58), 357
Warwick, B L, 350 (63), 357
Warwick, E J, 226 (195), 265, 365,
310 (93, 145), 320 (15, 93), 330,
332, 333
Watnabe, R, 258 (137), 263
Waterman, A, 196 (75a), 503
Watson, E J, D, 480 (126a), 505
Watson, M, 502
Watson R H, 292 (116, 147, 148), 294
(111), 304 (112), 332 333
Watson W. G, 531 (161), 537
Webber, A F, 22 (135, 176), 26, 174
(120), 183, 228 (186), 236 (186),
238 (186), 265
Webber, G F, 76 (152), 110

- Webster, H. D., 500 (129), 505
 Weeth, H. J., 234 (187), 236, 265
 Wehefritz, E., 526 (158), 537
 Weinberger, L. M., 212, 221
 Weiner, J. S., 35, 56
 Weinmann, J. P., 34 (68), 57
 Weinstein, C. L., 138 (161, 162), 151
 Weiss, L. P., 22 (151), 27
 Welch, J. A., 305 (67), 307 (67), 310 (67), 331
 Wellmann, O., 270 (78), 289
 Wells, L. J., 5, 12 (137, 148), 14, 15 (136, 148), 16 (68, 139, 140, 141, 142, 143, 145, 146, 147), 17 (139, 145, 146), 18, 25, 26, 27, 191 (200), 221, 495 (193), 498 (169), 497 (106), 504, 506, 507
 Welti, W., 336, 357
 Werbin, H., 497 (53), 503
 Werner, S. C., 490 (178), 508
 Wersäll, J., 21 (10), 23
 Werthesson, N. T., 429 (48), 432
 West, R. M., 94 (72), 108
 Westman, A., 76 (25), 82 (25), 106, 136 (54), 149
 Westman, J., 21 (10), 23
 Wharton, J. D., 123 (136), 151
 Wheeler, J. D., 22 (149), 27
 Wheeler, R. S., 167, 169, 181, 183
 White, A., 76 (168), 110
 White, D. E., 580 (16), 589
 White, E. P., 307 (9), 329
 White, P., 584 (55), 590
 White, W. E., 477 (194), 507
 Whitelaw, M. J., 346, 357
 Whitney, L. F., 364 (107), 366, 369 (111), 374, 376, 382, 388, 397
 Whitten, W. K., 199 (201), 221, 429 (63), 432, 483 (195), 485 (195), 507
 Whittlestone, W. G., 538 (165), 539 (165), 593
 Wiggins, E. L., 338, 350 (62), 357
 Wilde, W. S., 526 (49), 535
 Wiles, P., 517 (2), 559
 Wiley, T. L., 226 (119), 257, 263
 William, O. S., 291 (13), 297 (13), 330
 Wilhelm, A. E., 78 (169), 110
 Willett, E. L., 251, 252, 253, 265, 429 (61), 432, 470 (196), 507
 Williams, D. C., 140 (32), 148
 Williams, M. F., 131 (247), 154
 Williams, P. C., 79 (170), 110, 132 (248), 154
 Williams, R. E., 364, 395
 Williams, S. M., 295, 333, 501 (197), 507
 Williams, W. L., 277, 280, 289, 520 (160), 537, 544 (73), 554 (73), 591
 Wilson, E. B., 142, 154
 Wilson, K. M., 346, 357
 Wilson, W. K., 513 (159), 517 (159), 537
 Wilson, W. O., 169 (129), 183
 Wiltbank, J. N., 228, 246 (21), 257, 260, 285, 310 (150), 333
 Wilwerth, A. M., 167, 168, 189, 171, 183
 Wimsatt, W. A., 436, 449 (81), 450, 468
 Winchester, C. F., 169 (133), 172 (132), 183
 Windle, W. F., 496 (198), 507
 Wing, H. H., 517 (101), 537
 Winstone, N. E., 524 (53), 535
 Wintenberger, S., 294 (23), 299 (25, 27, 28, 29, 151, 152), 312 (28), 313 (23), 325 (23, 26), 329 (23), 330, 332, 333
 Wintenberger-Torres, S., 299 (153), 333
 Winter, C., 173, 183
 Winters, L. M., 2 (43, 150), 3 (150), 5 (150), 7 (150), 8 (150), 9 (150), 10 (150), 24, 27, 293 (45), 299 (45, 40), 330, 436, 444, 447, 462, 464, 465, 466, 467, 468
 Wishart, J., 517 (162), 537
 Wislocki, C. B., 22 (151), 27, 34 (92), 57, 445, 440, 447 (87), 450, 453 (87, 88), 454, 461, 463
 Wisnicky, W., 229 (41), 252 (38, 39), 261
 Witschl, E., 1 (153), 2 (152, 153), 3 (153), 27, 102 (171, 172), 110, 125 (219, 250), 154, 370, 397, 472, 495 (199), 507
 Witzigmann, J., 391, 394
 Wodzicki, K., 144 (111), 150, 277, 280 (46), 283, 284, 288
 Woehling, H. L., 310 (0), 341 (6), 355
 Wohlzogen, F. X., 91 (18), 96 (18), 106

- Wolfe, J M, 122, 149, 213 (202), 221,
 347, 348, 355, 357
 Wolff, E T, 175 (135), 176 (135), 183
 Wolff, J, 496 (110, 200), 504, 507
 Womack, E B, 495 (201), 507
 Wonder, D H, 76 (99), 89 (50), 107,
 108, 487 (61), 503
 Wood, W A, 373, 396
 Woodbury, R A, 524 (165), 526 (163),
 531 (164), 537, 538
 Woods, M C, 92 (173), 110, 253, 265
 Woolley, C W, 130 (251), 154
 Wooten, E, 87 (174), 110
 Wotton, R M, 401 (65), 432
 Wrenn, T R, 20 (92), 22 (93), 25,
 233 (132, 169), 230 (133), 238
 (133), 244 (169), 263, 264, 472
 (136), 500 (136), 505, 542 (151),
 574 (38), 581 (167), 586, 590, 593
 Wright, A, 481 (107), 482 (107), 504
 Wright, J B, 277 (12), 287
 Wright, J F, 385 (113), 397
 Wright, J C, 393 (114), 397
 Wright, P L, 434 (89), 468
- Y
- Yamada, H, 202 (180), 221, 258 (161,
 162, 163), 264
 Yamada, T, 192 (135), 219
 Yamamoto, H, 551 (162), 568 (162),
 571 (162), 577 (162, 163), 578
 (163), 593
 Yamamoto, T, 202 (185), 221, 258
 (161), 264
 Yamasaki, Y, 270 (69, 70), 277 (68,
 69), 289
 Yeates, N T M, 187, 188 (263), 191
 (203), 221, 292 (154, 155, 156),
 294 (155), 310 (154, 155), 333
 Yor, T S, 34 (93), 57
 Yoshimura, F, 202 (187), 221
 You, S S, 125 (40), 148
 Young, F G, 579, 580 (26), 581 (26,
 56), 589, 590
- Young, F W, 500 (129), 505
 Young, W C, 37 (88), 57, 165 (136,
 137), 183, 206, 216, 221
- Z
- Zacharias, L R, 193 (205), 221
 Zaffaroni, A, 487 (87), 504
 Zalesky, M, 191 (200), 221
 Zander, J, 118, 140 (253, 254), 154
 Zarrow, M X, 79 (136), 84 (175), 109,
 110, 118, 146 (3, 125, 126, 127),
 147 (127, 142), 147, 151, 152, 154,
 308, 332, 345, 355, 356, 357, 474
 (95, 138, 202), 486 (138), 489
 (95, 202), 491, 493 (202), 501 (95,
 138, 202), 504, 505, 507, 529 (109,
 167), 532, 535, 536, 538
 Zavadovski, M M, 326 (157, 158, 159,
 160, 101), 333
 Zeckwer, I T, 550 (169), 593
 Zehetner, F, 354 (70), 357
 Zeller, J H, 34 (80), 57, 337, 356
 Zietschmann, O, 29 (94), 51 (94), 57,
 437 (90), 441 (90), 468
 Zimmerman, D R, 350, 351, 357
 Zitrin, A, 210 (21, 22), 211 (21, 22),
 216
 Zondek, B, 34 (95), 57, 66, 77 (178),
 94 (3), 101 (178), 106, 110, 130
 (256), 154, 480, 487 (4), 493
 (204), 501, 507, 550 (169), 593
 Zuckerman, S, 2 (31, 32, 71), 13 (154),
 19 (41, 42, 82, 83, 84, 85, 86, 155,
 156), 24, 25, 27, 31, 43 (96), 46,
 47, 57, 134 (257, 258), 141, 143
 (67), 145, 149, 154, 187, 188, 189
 (46), 194, 209, 213, 214, 217, 219,
 220, 221, 231 (110), 249, 257
 (197), 261, 263, 265, 207 (35),
 208 (35), 274 (35), 278 (34), 280
 (35), 283, 288, 373, 378 (33), 393
 (33), 394, 397, 495 (205), 507
 Zupp, B A, 241 (198), 265
 Zwarenstein H, 125 (227), 153

Subject Index

A

ACTH, *see* Adrenocorticotropin

Accessory reproductive organs, *see* individual organs

effect of estrogen on, 134

lack of nutrients and atrophy of, 87

Adrenal

effect of removal on lactation, 554

in fetus, 496-497

interrelationship with thyroid, 177

relation to estrous cycle in cow, 249

role in pregnancy, 478-479

Adrenal cortex

androgens produced by, 114

estrogen in, 130

secretion of estrogens by, 133

Adrenal cortical hormones

effect upon lactation, 545

Adrenal extracts

effect on ovary, 173

Adrenal glands

histology and reproductive performance, 174

interrelation with thyroid, 177

role in reproduction, 172-177

Adrenal hormones

effect on egg production, 175

in normal female, 174

in normal male, 173

secretion rates of, 177

Adrenalectomy

effect on

estrous cycle in dog, 387

pregnancy, 479

replacement therapy and reproduction, 172

Adrenaline

effect on uterine motility in sow, 346

Adrenocorticotropin

effect on

deciduumata production, 173

lactation, 554

mammary development, 545

effect of pituitary transplantation on secretion of, 196

extra pituitary sources in pregnancy, 477-478

inhibition by adrenal and ovarian steroids, 207

liberation due to hypothalamic lesions, 188

placental secretion, 493

Adrenogenital syndrome, 175-177

Alkaline phosphatase

in reproductive tract of cow, 233

in uterus of cow, 244-245

in vagina of rat, 23

Allanto chorion

definition of, 445

differentiation in ruminants, 447

formation of, 446

necrosis at tip, 448

vesicles in, 461

Allantois, 445-451

development of

in carnivora, 450

in ewe, 447

in mare, 450

in sow, 446

formation of, 445

Amnion, 442-445

formation of, 442

in carnivora, 445

in cow, 444

in ewe, 444

in mare, 444

in sow, 444

function of, 443

hippomanes and, 443-444

Ampulla

development of, 30

glands of, 48

Anatomy

of female reproductive organs, 1-27,

see also individual organs

in cow, 229-231

of male reproductive organs, 29-57,

see also individual organs

Androgens, 418

Androgens, 110-129, *see also* specific

androgenic compounds and Gonadal hormones

assay of, 127-129

blood levels in men and women, 110

chemical assay, 128
 chemical structure, 112
 control of pituitary by, 79
 effect on

chickens, 127
 coagulating gland, 124
 embryo, 127
 female, 125-127
 female preputial glands, 126
 fructose secretion, 123
 gonadotropin secretion, 115
 lower vertebrates, 125
 male reproductive tract, 122-125
 mammary development, 546
 mating behavior in female, 206
 ovary, 126
 penis, 122
 perineal muscle of rat, 122
 preputial glands, 124
 prostate, 45, 123
 scrotum, 123
 seminiferous tubules, 125
 sexual desire in female, 126
 testis, 69, 79, 124, 125
 vagina of rat, 126
 gonadotropic action, 125
 inhibitory effect on estrogen, 134
 international unit of, 127
 methods of administration, 116
 ovulation in toad induced by, 125
 physiological actions, 115
 produced by adrenal cortex, 114
 relation to 17-ketosteroid excretion, 129
 role during breeding season, 119-120
 role at puberty, 119-120
 and sex reversal, 127
 sources of, 114
 and x-zone of adrenal, 123
 Androstenedione
 chemical structure of, 112
 Androsterone
 blood levels of, 115
 chemical structure of, 112
 Anestrus, 140
 duration in dog, 364
 gonadotropic activity in mare during, 271
 in mare, 269
 ovary of mare during, 269, 278

Anterior pituitary, *see* Pituitary, anterior
 Antifertilizin, 410
 Antigonadotropins, 77-78
 Antihormones, *see* Antigonadotropins
 Arthus phenomenon, 12
 Assay

of androgens, 127-129
 of estrogens, 135-137
 of gonadotropins, 90-100
 of progesterone, 139-140
 of relaxin, 147
 Augmentation, *see* Synergism

B

Behavior, effect of androgen on
 in the male, 115
 Blastocoele, formation of, 421
 Blastocyst
 nidation of, 473
 orientation of
 in carnivora, 437
 in sow, 436
 spacing of, 434, 470
 Blastomeres, differentiation of cell types
 in, 424
 Bleeding, proestrous, in dog, 378
 Blood, chemical changes during cycle in
 dogs, 366
 Blood supply to genital organs in dogs,
 369
 Boar, prostate of, 42
 Body growth from birth to puberty in
 dog, 362-363
 Breeding
 optimum time in cow, 227
 Breeding efficiency
 effect of nutrition in mare on, 275
 seasonal effects in mare on, 270
 Breeding program, in mare, 285-287
 Breeding season, *see also* Sexual season
 effect of
 domestication in mare on, 267-268
 gonadotropic activity in mare during,
 271
 in jennet, 270
 in mare, 267-271
 role of androgens in, 119
 Bulbourethral glands, 41
 anatomy of, 48
 development of, 30
 Bull, prostate of, 42

C

- Capacitation
 in bovine spermatozoa, 227
 of spermatozoa, 408
 time required for, 409
- Caruncles
 location of, 457
 number in various animals, 457
 shape of, 457
- Castration, *see also* Ovariectomy
 age response to hormone therapy after, 207
 androgen excretion following, 121
 effects of, 120-122
 effect on
 anterior pituitary, 122
 chicken, 121
 fetal rabbits and mice, 16-18
 mammals, 121-122
 secretion of urinary gonadotropin, 122
- Cat, estrous cycle of, 144
- Cell types, embryonic differentiation of, 421
- Cervical stimulation, prolongation of life of corpora lutea by, 74
- Cervix
 anatomy of, in dog, 371
 carcinoma of, 12
 cement substance in, 22
 cyclic changes
 in cow, 239
 in dog, 378-379
 in mare, 281-282
 endometrial rugae of, 7, 23
 hormonal control of mucus secretion in cow, 499
 precollagen in, 22
- Chemotaxis, 409
- Chicken
 effect of
 androgen on, 127
 castration on, 121
 hypothyroidism on size of ovary, 172
- Chorion, 415-451, *see also* Placenta
 definition of, 445
 "rosettes" of, 461
- Cleavage
 blastomere formation, 419
 mechanisms of, 419
 rate of, 426
 volume changes during, 426
 of zygote, 418
- Clitoris, 6
- Coagulating gland, effect of androgen on, 124
- Cockerels
 effect of hypothyroidism on testis of, 170
 influence of thyroid hormone on semen production in, 166-168
- Coitus, in dog, 392
- Conj vasculosi, 36
- Corpus luteum
 cell types in cow, 234
 cyclic changes during estrous cycle in sow, 342-343
 cysts in bovine, 233
 development in dog of, 382-383
 effect of removal in cow, 250, 499
 in ewe, 298
 formation of, 142
 in cow, 233
 in dogs, 374
 in mare, 279
 life of, 142, 145
 in monkey, 19
 during pregnancy, 472
 of pregnancy in dog, 382
 in rat, 19
 regression in dog, 383
 size in cow, 233
 in sow, 342-343
- Cortisone
 effect on
 deciduumata production, 173
 mammary involution, 545
- Cotyledons, *see also* Caruncles
 in cattle, 22
 in deer, 22
 formation of
 in cow, 458
 in sheep, 458
 location, 457
- Cow
 age at puberty, 224
 anatomy of
 reproductive organs, 229-231
 uterus, 21
 cell types in corpus luteum of, 234

- chemical composition of follicular fluid, 231
 corpus luteum formation in, 233
 cyclic changes
 in cervix, 239
 in endometrium, 236
 in ovary, 231-234
 in uterus, 235
 in vagina, 239
 in vaginal mucus, 242
 delta cells in anterior pituitary of, 256
 duration of estrus, 225
 effect of corpus luteum removal during pregnancy, 499
 estrous behavior in, 225
 estrous cycle of, 223-265
 follicular atresia in, 231
 gestation length
 breed differences in, 513
 heritability of, 512
 paternal influence on, 511
 relation to age of dam, 518
 seasonal effects, 514
 growth of follicle in, 232
 initiation of estrus following parturition in, 228
 involution of uterus in, 229
 length of estrous cycle in, 225
 metestrous bleeding in, 228
 optimum time for breeding of, 227
 ovulation in, 232
 ovulation induced in, 81
 prolonged gestation in, 520
 clinical and pathological aspects of, 521-522
 size of reproductive tract, 230
 thyroxin secretion rate of, 158-160
 time of insemination in relation to fertility in, 227
 time of ovulation, 226
 uterus of, 230
 vaginal smear in, 241
 Cowper's glands, *see* Bulbourethral glands
 Cumulus oophorus, 400
 hyaluronic acid in, 411
 penetration by sperm, 411
 Cyclopentanoperhydrophenanthrene, nucleus of, 112
 Cystic ovary
 in dog, 366
 in hypothyroid mice following gonadotropin injection, 162
- ## D
- Decidual cells in endometrium of mare, as possible source of equine gonadotropin, 498
 Deciduoma, progesterone and, 137
 Dehydroisoandrosterone, blood levels of, 115
 Delta cells, in anterior pituitary, 247, 256
 Deoxyribonucleic acid
 quantity in nucleus, 416
 synthesis by pronuclei, 415
 Deutoplasm, 427
 Development of male reproductive organs, 30-31
 Diestrus
 definition of, 140
 duration in mare of, 274-276
 following parturition in mare, 275
 Diet
 effect of, in reproduction, 86 88
 Dog
 age at puberty, 362
 corpus luteum of, 374
 cyclic changes
 in genital organs, 371-379
 in ovary, 372-376
 in oviduct, 377
 in uterus, 378
 detection of estrus, 366
 development of corpus luteum, 382-383
 duration of gestation in, 393
 effect of nutrition on estrous cycle in, 384
 hormones on estrous cycle, 388-390
 season on estrous cycle, 384
 embryonic differentiation of gonads, 360
 estrous cycle of, 359-397
 anatomical changes in genital organs 367-381
 factors influencing 381-392
 fecundity of, 392
 fetal reproductive system in, 361
 mammary gland tumors in, 391

- migration of fertilized ova through oviduct in, 360
- ovary at birth of, 361, 362
- parturition in, 393
- prepuberal development of ovary in, 363
- prepuberal growth in, 362
- proestrous bleeding in, 378
- prostate of, 43
- pseudopregnancy in, 383
- sexual behavior of, 365-368
- suspension of internal genital organs in, 367
- vaginal smear of, 379-381
- Duck, influence of thyroid hormone on semen production in, 166
- Ductus deferens, anatomy of, 37

E

- Ecology, effect on estrous cycle in dog, 385
- Ejaculatory duct, 17
- Embryo
 - of bovine, 3
 - chemical constituents of, 429
 - development of
 - in cow, 462
 - in ewe, 462
 - differentiation of gonad in dog, 360
 - early physiology of, 428
 - effect of
 - androgens on, 127
 - castration on, 127
 - estrogen on, 134-135
 - formation of morula, 420
 - implantation in dog, 360
 - implantation of, 427
 - internal migration of, 429
 - in vitro culture of, 429
 - mortality of, 470
 - nutrition of, 434
 - oxygen consumption of, 429
 - polyploidy in, 417
 - rate of transport of, 428
 - transplantation of, 429
 - transport in oviduct of, 428
 - Wolffian duct in chick, 11
- Embryotrophe, 434
- Endometrial cups
 - formation of, 455, 497
 - function of, 498
 - hormone content of, 498
 - secretion of gonadotropin by, 22, 493, 497, 498
- Endometrium
 - cyclic changes
 - in cow, 236
 - in monkey, 22
 - in women, 22
 - effect of
 - placental estrogen in newborn on, 12
 - progesterone in newborn on, 12
 - histochemical studies of cow, 22
 - pregnancy changes in, 454
- Environment
 - effect on
 - estrous cycle in dog, 384
 - sexual season of ewe, 292
 - gonadotropin secretion as affected by, 186, 270-271
 - hypothalamus in reproduction and, 188-191
 - temperature effect on reproductive cycle, 190-191
- Environment, internal, influence on gestation length, 518
- Epididymis
 - anatomy of, 37
 - development of, 30
- Epinephrine
 - blocking effect on lactation, 558
 - effect on uterine motility in cow, 246
- Equilin, chemical structure of, 113
- Estradiol
 - blood levels in cow, 254
 - chemical structure of, 113
 - international unit of, 135
- Estradiol-17 β , synthesis in pregnancy, 480
- 17-ethinyl-5(10)-estraeneolone, 113
- Estriol, chemical structure of, 113
- Estrogens, 129-137, *see also* individual compounds, and gonadal hormones
 - action on vaginal slices in tissue culture, 117
 - in adrenal cortex, 130
 - assay of, 135-137
 - biosynthesis from acetate, 114

- blood levels of, 117
- breeding efficiency following treatment with, 577
- chemical assay of, 136
- chemical caponization by, 133
- chemical hypophysectomy by, 133
- chemical structure of, 113
- control of pituitary by, 79
- cornification of vagina by, 130
- in cow ovary, 344
- cyclic secretion of, 129
- effect of
 - prolonged treatment with, 131
- effect on
 - bleeding in dog 387
 - cervix of cow, 246
 - embryo, 134-135
 - embryos of birds, 135
 - embryos of lower vertebrates, 134
 - embryos of mammals, 135
 - estrous cycle in dog, 389
 - estrous cycle of sow, 354 355
 - female reproductive tract, 130-132
 - female sexual behavior, 117
 - gonadotropin secretion by pituitary, 132
 - growth of mammary gland, 540
 - initiation of parturition, 529
 - male, 133-134
 - male accessory reproductive organs, 134
 - mammary gland, 132
 - mammary gland of male, 134
 - mating behavior in male, 206
 - milk yield, 584 589
 - ovary, 132, 271
 - oviduct, 132
 - phosphatase in cow uterus, 244
 - pituitary gland, 117
 - pituitary of mare, 286
 - prolactin content of pituitary, 549
 - pubic symphysis in male, 133
 - secondary sex characters in male, 133
 - semen 133
 - sex skin in primates, 116
 - testis in newborn opossums 15
 - thyroid gland, 134
 - udder congestion 588
 - uterine motility, 131
 - uterus, 131
 - uterus of cow, 244
 - vagina of castrate, 131
 - excretion in ewe of, 307
 - in female urine, 118
 - feminization of male with, 133
 - in fish, 130
 - functions of, 472
 - function of, during pregnancy, 475
 - glycogen deposition in vagina and, 131
 - to induce heat in ewe, 313
 - infertility and, 131
 - influence on mating behavior, 205
 - inhibition by androgen, 134
 - interaction with progesterone, 137
 - international unit of, 135
 - intravaginal test for, 136
 - isolation from organs of, 114
 - lactation depression by, 559
 - in male urine, 34
 - mammogen I secretion and, 544
 - methods of administration, 118
 - nymphomania caused by, 577
 - physiological actions of, 116 117
 - physiological functions in cow, 245
 - in placenta, 492
 - production by stallion testis, 72
 - and progesterone ratio
 - in growth of mammary gland, 542
 - in parturition initiation, 530
 - protein anabolic effect in ruminants of, 116
 - relaxation of symphysis pubis by, 147
 - requirements during pregnancy, 83
 - role at puberty, 129
 - role in initiation of lactation, 559
 - secretion by adrenal cortex, 133
 - sterility in male and, 133
 - synergism with relaxin, 532
 - synthesis during pregnancy of, 180
 - in testis 34
 - titers during pregnancy of, 482
 - urinary excretion in cow of, 253
 - urinary titers during pregnancy of 183
 - vaginal mitoses and, 136

Estrone

 - chemical structure of 113
 - international unit of, 135

Estrous cycle

 - in cat 111
 - in cow 223 265

- cyclic changes in
 - activity of sow, 349
 - alkaline phosphatase in cow, 233
 - anterior pituitary of sow, 347-349
 - corpus luteum of sow, 342-343
 - genital organs in dog, 367-381
 - oviduct of cow, 21
 - oviduct of sheep, 21
 - sow, 341-349
 - uterus of sow, 345-346
 - vagina of sow, 346-347
 - vulva of sow, 349
 - in dog, 359-397
 - ecological influence in dog on, 385
 - effect of
 - adrenalectomy in dog on, 387
 - breeding in dog on, 364
 - estrogens in dog on, 389
 - estrogens in sow on, 354-355
 - estrogens in spayed dog on, 387
 - gonadal hormones in sow on, 353-355
 - gonadotropins in dog on, 387-388
 - gonadotropins in mare on, 286
 - gonadotropins in sow on, 352-353
 - hypophysectomy in dog on, 386
 - ovarian grafts in spayed dogs on, 387
 - ovariectomy in dog on, 386
 - oxytocin on, 358
 - oxytocin in cow on, 253
 - progesterone in dog on, 390
 - progesterone in sow on, 353-354
 - supplementary progesterone in cow on, 250
 - experimental modification of, in sow, 352-355
 - in ewe, 291-328
 - in ferret, 144
 - follicular phase of, 141
 - general considerations of, 140-145
 - in goat, 328-329
 - hormonal regulation of, 141
 - hormone content in ovary of sow during, 344-345
 - initiation following parturition in cow of, 225
 - length of
 - in cow, 225
 - in dog, 364
 - in ewe, 295
 - in mare, 271-272
 - in sow, 338-339
 - luteal phase of, 141
 - in mare, 271-277
 - mechanisms controlling in mare, 270-271
 - methods of altering in cow, 250
 - in mouse, 144
 - nutritional influence in dog on, 384
 - pathological types in dog of, 390-392
 - in rabbit, 144
 - in rat, 144
 - relation of gonadotropins and type of, 145
 - seasonal effect in dog on, 384
 - seasonal variation in mare of, 269
 - species differences with respect to bleeding, 143-144
 - in sow, 335-357
 - theory of controlling mechanisms of, 79-80
 - types of, 144-145
 - vaginal changes in dog during, 379
 - variation in number of ova in rat ovary during, 19
 - X-irradiation influence in dog on, 385
- Estrus
- abnormalities in dog of, 390
 - behavior in cow during, 225
 - control in ewe of, 312-323
 - constant, induced by hypothalamic lesions, 198
 - definition of, 140
 - detection of
 - in dog, 366
 - in mare, 285
 - duration of
 - in cow, 225
 - effect of coitus in sow on, 340
 - effect of sexual age in sow on, 339
 - in ewe, 296
 - in heifers, 226
 - in mare, 272-274
 - in sow, 339-340
 - effect of lactation in sow on, 340-341
 - hormonal induction in ewe, 312-323
 - inhibition by progesterone in cow of, 244

- manifestations of
 - in dog, 365-366
 - in sow, 341
- ovulation time in sow related to, 349-350
- following parturition in mares, 276-277
- during pregnancy in ewe, 501
- produced in ovariectomized cows, 244
- progesterone and, 138
- time in relation to
 - parturition in sow, 340
 - weaning in sow, 340-341
- seasonal variation
 - in jennet of, 274
 - in mare, 273-274
- Ethinyltestosterone, 113
- Ewe
 - control of
 - changes in reproductive tract, 323-325
 - estrus in, 312-323
 - ovulation in, 309-312
 - corpus luteum in, 298
 - cyclic changes
 - in oviduct, 298
 - in reproductive tract, 296-304
 - in uterus, 299
 - in vaginal smear, 300-304
 - estrogen excretion in, 307
 - estrous cycle of, 291-328
 - estrus induced with progesterone in, 313
 - "flushing" in, 297
 - follicular growth in, 297
 - gestation length
 - heritability of, 512
 - nutritional effect on, 515
 - relation to age of dam, 519
 - seasonal effect on, 515
 - gonadotropin content in pituitary of, 305-307
 - hormonal control of ovulation in, 325-328
 - hormonal induction of estrus in, 312-323
 - maintenance of pregnancy after ovariectomy in, 501
 - neural control of ovulation in, 310
 - ovulation in, 296-298
 - ovulation induced with gonadotropins in, 81, 310-312
 - ovum transport in, 298
 - progesterone content in blood, 308
 - progesterone levels during pregnancy in, 501
 - sexual season of, 292
 - "silent" estrus in, 294
 - sperm transport in, 299
 - superovulation with gonadotropins in, 325-328
- Eye
 - receptor in reproductive photoperiodism of, 188-189
- F
- FSH, *see* Follicle stimulating hormone
- Fallopian tubes, *see* Oviduct
- Fecundity
 - in dog, 392
- Female reproductive organs, *see also* individual organs
 - anatomy of, 1-27
 - in cow, 229-231
 - cyclic changes in, 142-145
 - cyclic changes
 - in mare, 281-284
 - development of, 1-13
 - effect of androgens on, 126
 - in goat, 328-329
 - histological changes in mare of, 282-284
 - hormonal control in ewe of, 323-325
 - transport of sperm in, 407
- Ferret
 - estrous cycle of, 144
- Fertility
 - in dog 392
 - effect of
 - age in dog on, 376
 - progesterone on, 251
 - in foal heat, 277
 - hormone interrelations and, 84-80
 - puberty and, 129
 - return to postpartum in cow, 229
 - seasonal changes of, in mare, 268
- Fertilization, 110-118
 - abnormalities of, 417
 - activation of ovum and, 414
 - block to polyspermy and, 113

- definition of, 410
 effect of
 number of sperm in oviduct, 227
 sperm concentration on, 409
 time of insemination in cows, 227-228
 penetration of sperm through the cumulus oöphorus, 411
 physiological mechanisms of, 408-418
 polyspermy and, 409
 pronucleus formation, 414
 role of cumulus oöphorus, 409
 site of, 405
 syngamy and, 414
 time relations of, 417
 in vitro, 418
 zona lysin and, 412
 zona pellucida and, 411
 zona reaction following, 412
- Fertilization cone**, 412
- Fertilizin**
 in mammals, 410
- Fertilizin-antifertilizin reaction**, 410
- Fetal ovaries**
 production of sex hormones by, 16
- Fetal pituitary**
 effect on parturition, 531
- Fetal testis**
 influence of injected gonadotropin on, 16
- Fetus**
 adrenal gland activity in, 496
 of bovine, 4, 6
 development of, 462
 effects of
 castration on, 495
 hormones on, 495
 endocrine functions of, 495-497
 estimating age of, 461-468
 factors affecting size at birth, 463
 hormones in gonads of, 495
 mortality of, 470-471
 number and size affecting gestation length, 516
 prolonged, abnormalities of, in Guernseys, 521
 sex of, influencing gestation length, 517
 size in prolonged gestation in Guernseys, 521
- thyroid of, 496
 weight, age formula of, 464
- Flushing**
 effect on ovulation rate in sow, 350-351
 in ewe, 297-298
- Foal heat**, 276-277
- fertility in**, 277
- Follicle**, *see* Ovarian follicle
- Follicle stimulating hormone**, *see also* Gonadotropin(s), pituitary
 assay of, 91-93
 effect on
 estrous cycle, 141
 hypophysectomized rats, 67-72, 81-83
 in hypothyroidism, 161
 infundibular control of secretion, 197
 pituitary content during pregnancy, 476
 purity of, 72
 ratio to luteinizing hormone
 in body fluids, 102-104
 in pituitary, 102-104
 secretory control of, 78-80, 196-198
 species differences in pituitary content of, 145
- Freemartin**
 development of, 495
 Lillie's theory of, 15, 127, 495-496
- Fructose**
 effect of androgen on secretion of, 123
- G**
- Genetic sex**, recognition of, 1
- Genital organs**, *see also* Reproductive organs
 blood supply in dog of, 369
 cyclic changes in dog of, 371-381
 prepuberal development in dog of, 360-364
 suspension in dog of, 367
- Germinal epithelium**, as source of ova during maturity, 19
- Germinal vesicle**, 401
- Gestation**, *see also* Pregnancy
 duration of, in dog, 393
 prolactin concentration in pituitary during, 551
- Gestation length**, 509-522, *see also* Prolonged gestation

- breed differences
 - in beef cattle, 513
 - in goats, 513
 - in rabbit, 513
- in breeds of domestic animals, 510-511, 513
- effect of
 - environment on, 513-519
 - fetus in horse on, 512
 - hereditary factors on, 511
 - number and size of fetuses on, 516
 - order of pregnancy on, 518
 - paternal genetic factors on, 511, 513
- factors affecting, 509-519
- heritability of
 - in cow, 512
 - in ewe, 512
 - in mare, 512
- internal environmental influences, definition of, 516
- nutritional effect on
 - in ewe, 515
 - in mare, 515
- relation to age of dam
 - in cow, 518
 - in ewe, 519
 - in goat, 519
 - in mare, 519
 - in sow, 519
- seasonal effects
 - in cow, 514
 - in ewe, 515
 - in goat, 515
 - in mare, 514
- Glycogen
 - cyclic changes in uterus of cow, 236
 - deposition in vagina following estrogen, 131
 - in ovarian follicle, 233
 - in relation to estrous cycle, 233
- Goat
 - effect of ovariectomy on, 500
 - estrous cycle of, 328-329
 - gestation length
 - breed differences in, 513
 - relation to age of dam in, 519
 - seasonal effect on, 515
 - ovulation induced in, 81
 - reproductive activity in, 328-329
- Goitre, endemic, effects of, in domestic animals, 157
- Goitrogens
 - hypothyroidism and, 157, 161-162, 165, 170-172
- Gonadal hormones, 111-153, *see also* individual hormones
 - blood levels of, 115-119
 - dosage of, 115-119
 - effect on estrous cycle of sow, 353-355
 - estrous cycle and, 140-145
 - general actions of, 115-119
 - mammary gland growth and, 540
 - source of, 114-115
 - types of, 112-113
- Gonadotropin(s), *see also* individual hormones
 - antagonism between, 72-74
 - bioassay of, 90-100
 - bioassay in urine of nonpregnant human, 91
 - biological properties in urine of nonpregnant human, 97-100
 - in body fluids
 - of nonpregnant animals, 90
 - during pregnancy, 88-90
 - relation to pituitary gonadotropins, 100-106
 - chemical fractionation of, 75
 - content in anterior pituitary of sow, 348-349
 - duality of, 67
 - effect on estrous cycle
 - in dog, 387-388
 - in mare, 286
 - in sow, 352-353
 - effect on ovary
 - of menses ewe, 271
 - of calf, 251
 - of cow, 252
 - in humans, 78
 - induction of ovulation in cow, 253
 - effect on secretion of
 - by androgens, 115
 - by estration, 122
 - by environment, 186-191
 - by estrogens, 132
 - by lesions of hypothalamus, 191-192
 - estrous induction in ewe with, 319
 - influence on fetal testes, 16

- levels in nonpregnant human, 98-100
- neural control of secretion, 186-203
- ovulation induced in ewe with, 310-312
- physicochemical properties of, 78
- in prenatal life, 8
- purity of, 75-76
- relation of pituitary and urinary gonadotropins, 100-106
- relation to type of estrous cycle, 145, 270-271
- relative potency in hypophysectomized rat, 69
- in rhesus monkey, 90
- role in reproductive processes, 49-110
- seasonal ratios in mare, 271
- specificity of, 77
- survival in the circulation of, 101
- synergism between, 72-74
- urinary concentration during menstrual cycle, 80
- Gonadotropin complex, 67-75
- Gonadotropin, equine
 - assay of, 96-97
 - biological properties of, 88, 494
 - blood levels during pregnancy, 88-90, 488-489
 - chemistry of, 493
 - effect on
 - estrous cycle of dog, 388
 - ovary of pregnant mare, 497
 - tubules of testis, 89
 - ovulation induced by
 - in cows, 81-82
 - in ewes, 81
 - in goats, 81
 - in mice, 82
 - in rats, 82
 - in sows, 82
 - in women, 82
 - purification of, 88
 - source of, 493, 497-498
 - urinary levels of, 488
- Gonadotropin, human chorionic
 - assay of, 91-96
 - biological properties of, 88, 494
 - chemistry of, 493
 - effect on
 - estrous cycle in dog, 388
 - fetal rat ovaries, 17
 - tubules of testis, 69
- levels of, 487
- ovulation induced by
 - in cows, 81
 - in mares, 288
 - in mice, 82
 - in rats, 82
 - in women, 82
- purification of, 88
- source of, 493
- in urine during pregnancy, 88-90
- Gonadotropin(s), pituitary, 59-110, *see also* Follicle stimulating hormone, Luteinizing hormone and Prolactin
 - assay of, 91-94
 - chemical fractionation of, 75
 - content in ewe of, 305-307
 - drugs causing release of, 200-201
 - effect on
 - ovaries of fetal rats, 17
 - hormonal regulation of secretion, 78-80
 - level during pregnancy, 478
 - mechanism of release, 199
 - neural control of secretion, 80
 - ovulation induced by
 - in calves, 82
 - in cows, 81
 - in mice, 82
 - in monkey, 82-83
 - in rats, 82-83
 - in women, 82-83
 - relations to gonadotropins in body fluids, 100-108
 - species specificity of, 77-78
- Gonadotropin(s), placental
 - function of, 494
- Gonads, *see also* Ovary and Testis
 - activity after hypophysectomy, 61-66
 - development in horse fetus of, 498
 - embryonic differentiation in dogs, 360
 - hormone content in horse fetus of, 499
 - mesenchymal primordium, 3
 - role in sex differentiation, 13-18
 - suspensory ligament of, 5
- Gonads, embryonic
 - hormone secretion by, 127
- Gonads, fetal
 - hormones in, 495
- Graafian follicle, *see* Ovarian follicle

- Growth hormone
 effect on
 lactation, 578-581
 mammary gland growth, 545
 milk yield, 579
 role in pregnancy of, 477
 Gubernaculi, 12
 Gynogenesis, 418
- ## H
- HCG, *see* Gonadotropin, human chorionic
 Heat, *see* Estrus
 Hemotrophe, 434
 Heparin
 in human endometrium, 22
 tyrosine and tryptophan in, 22
 Hippomanes, 444
 Histotrophe, 434
 Hooker-Forbes test, 139
 Hyaluronic acid
 in cumulus oophorus, 411
 Hyaluronidase
 in spermatozoa, 411
 Hypersexuality, effect of brain lesions on, 210-211
 Hyperthyroidism, *see also* Thyroprotein, Thyroid hormone and Thyroxine
 definition of, 158
 effect on
 puberty in mice, 161
 response to gonadotropins, 161-162
 semen production of rams, 164
 Hypogastric nodes, 20
 Hypophysectomy
 atrophy of the gonads following, 60
 effect on
 estrous cycle in dog, 386
 lactation, 547
 pregnancy, 474, 477
 reproductive system, 60-66
 in fetal rats and mice, 16
 gonadal activity following, 61-66
 light response affected by, 188
 ovarian regression following, 60
 substitution therapy after, 66
 testicular regression following, 60-61
 Hypophysectomy, chemical
 by estrogens, 133
 Hypothalamus
 dissected of, affecting gonadotropin secretion, 191-192
 effect on parturition, 214-215
 in environmental control of reproduction, 188-191
 lesions of
 gonadotropin secretion and, 191-192
 mating behavior and, 208-209
 puberty and, 213
 reproductive response to light and, 188
 lutemizing hormone release control by, 198-200
 prolactin secretion independent of, 201
 role in parturition of, 531
 Hypothyroidism, *see also* Thiouracil and Thyroidectomy
 effect on
 estrous cycle of bovine, 163
 gonadotropin secretion, 161
 menstrual cycle of monkeys, 163
 ovary of birds, 172
 response to gonadotropins, 161-162
 testes of birds, 170-171
- ## I
- ICSH, *see* Luteinizing hormone
 Implantation, 436-438
 delayed, 434
 due to season, 514
 in dog, 360, 392
 methods of, 434
 time of, 428
 in cow, 436
 in mare, 437
 in sheep, 436
 in sow, 436
 Inbreeding
 effect on puberty in sow of, 337-338
 Infertility
 estrogen and, 131
 hormonal interrelations in, 84-86
 Inner cell mass, 424
 Insemination, time in relation to fertility
 in cow, 227
 International unit
 of androsterone, 127
 of equine gonadotropin, 91
 of estradiol, 135
 of estrone, 135
 of human chorionic gonadotropin, 91
 of prolactin, 91

- Interstitial cell stimulating hormone, *see*
Luteinizing hormone
- Interstitial cells of testis, 69
development of, 2
source of androgens and, 34
- Interstitial tissue of ovary
comparative studies of, 20
derivation from theca interna, 19
esterified cholesterol in, 20
lipid granules in, 19
of rat, 19
- Intoxication
due to incompatibility in parabiosis,
528

J

- Jennet
breeding season of, 270
seasonal variation in length of estrus
in, 274

K

- 17-Ketosteroids
blood levels of, 115
effect of oxytocin on excretion of, 258
excretion during childhood, 119-120
in human female, 125
relation to androgen secretion, 129

L

- LH, *see* Luteinizing hormone
- Labor
induced in sow with oxytocin, 531
oxytocin release during, 527
in prolonged gestation, 521
stages of, 523-526
- Lactation
blockage by epinephrine of, 558
curve following experimental induction of, 569
depression by estrogen of, 559
effect of
adrenal cortical hormones on, 554
adrenalectomy on, 554
adrenocorticotropin on, 554
different hormone ratios on curve of,
569-576
hormones at various stages on, 578
hypophysectomy on, 547
induction on health, 577

- nervous system on, 555
neurohypophysis on, 559
pancreas on, 554
parathyroid on, 555
posterior pituitary on, 558
somatotropin on, 578-581
suckling stimulus on maintenance
of, 555
effect on estrous cycle in sow, 340-341
experimentally induced, characteristics
of milk, 565
experimental induction of, 565-577
failure in hypophysectomized rats, 84
galactopoietic substances and, 578
hormonal requirements for, 546-593
hormones used for experimental induction of, 565
increase by hormones of, 577-589
initiation and maintenance
by anterior pituitary, 547
by prolactin, 548
mechanism of suckling stimulus and,
558
mechanisms controlling initiation at
parturition, 559-565
Meites-Turner theory of, 560-564
method of experimental induction of,
566
neural factors controlling, 215-216
in partially developed mammary system,
542
role of estrogens in initiation of, 559
- Leydig cells, *see* Interstitial cells of testis
- Light
duration necessary for reproductive
stimulation, 188
effect of wave length on reproduction,
187
effect on
ovarian function in mare, 270
pituitary activity of mare, 270
sexual season in ewe, 292
gonadal regression inhibited by, 187
hypophysectomy affecting response to,
188
light-dark alteration, effect of, 188
metabolism increased by, 190
reproductive cycle control by, 186-191
threshold for stimulation of reproductive
activity, 188

- Light reception
 - in birds, 189
 - in mammals, 188-189
- optic nerve in mammals and, 188-189
- ventral nuclei in lateral geniculate body and, 189
- Lillie's freemartin theory, 15
- Lobuli epididymidis, *see* Coni vasculosi
- Luteinizing hormone
 - assay of, 92-94
 - control of secretion of, 198-201
 - effect in hypophysectomized rats, 67
 - estrous cycle and, 141
 - pituitary content during pregnancy of, 476
 - ratio to FSH
 - in body fluids, 102-104
 - in pituitary, 102-104
- Luteotropin, *see* Prolactin

M

- Male accessory reproductive organs
 - influence of a male sex hormone on, 16
 - response to sex hormones during childhood, 119
- Male accessory sex glands
 - anatomy of, 41-48
 - arterial supply to, 42
- Male hormone, *see* Androgens
- Male reproductive organs, *see also* individual organs
 - anatomy of, 29-57
 - development of, 30-31
 - postnatal anatomy of, 31-55
- Mammals, effects of castration on, 121-122
- Mammary gland, 6, 539-569
 - adrenal cortical influence on development of, 545
 - derivation and development of, 5, 539-546
 - effect of
 - androgens on development, 546
 - anterior pituitary hormone on growth, 542-545
 - estrogens on, 132
 - estrogen on ducts, 540
 - estrogen progesterone mixtures on, 540
 - gonadal hormones on growth of, 540
 - growth hormone on growth of, 545
 - hormones in hypophysectomized, ovariectomized rats on, 544
 - placenta on development of, 546
 - progesterone on, 139
 - thyroid on development of, 545
 - estrogen-progesterone ratio for growth of, 542
 - growth during lactation, 578
 - growth and lactation antagonism, 560-563
 - growth period during pregnancy of, 491
 - growth in pseudopregnant dog of, 383
 - hormones required for growth of, 540-546
 - involution in dog, 384
 - involution prevention by cortisone acetate, 545
 - lactation in partially developed system, 542
 - pregnancy changes in, 490
 - progesterone-estrogen ratios for full development of, 491
 - prolactin and growth of, 544
 - relaxin and, 147
 - size, effect on milk produced by, 540
 - species differences in growth requirements of, 139
 - supernumerary pair of nipples, 7
 - tumors in dog, 391
- Mammogen I
 - estrogen as stimulator of secretion, 544
- Mammogen II
 - estrogen-progesterone stimulation of secretion, 544
- Mare
 - breeding program for, 285-287
 - cyclic changes
 - in cervix, 281-282
 - in mucus, 281
 - in ovary, 279-280
 - in uterus, 282
 - in vagina, 281
 - diestrus, 274-276
 - following parturition, 275
 - effect of
 - domestication on breeding season, 267-268
 - estrogen on ovary, 271

- estrogen on pituitary, 286
 - gonadotropin on estrous cycle, 286
 - gonadotropin on ovulation, 286
 - nutrition on breeding efficiency of, 275
 - estrus in, 285
 - following parturition in, 276-277
 - foal heat of, 276-277
 - gestation length
 - effect of nutrition on, 515
 - heritability of, 512
 - histological changes in reproductive tract, 282-284
 - mating behavior, 284-285
 - ovary of, 277-279
 - pregnancy diagnosis in, 89-90
 - prolonged gestation in, 520
 - pseudoestrus in, 269
 - seasonal changes
 - in breeding efficiency, 270
 - in fertility, 268
 - in gonadotropic activity, 271
 - in length of estrus, 273-274
 - silent estrus in, 285
 - vaginal smear of, 283
 - viability of ovum in, 286
 - viability of sperm in reproductive tract of, 286
- Mating behavior, 203-211
- brain areas involved with, 208-211
 - decortication and male sex activity, 209-210
 - in dog, 392
 - effect of
 - castration on, 205
 - hormone administration on, 207
 - hormonal control of, 205-211
 - hypersexuality due to brain lesions, 210-211
 - in mare, 284-285
 - nonspecific action of hormones on, 206
 - physiological mechanism of gonadal hormones, 207-211
 - reflex patterns of, 204-205
- Maturation of ovarian follicle in dog, 372
- Mediastinum, 32
- Medullary cords
 - in human ovary, 9
 - ovarian, 15
- Meiosis
 - phases of, 402

Mesonephros, 4

Mesothelium, 11
 - formation of ova from, 5
 - secondary sex cords from, 4

Metabolism
 - light effect on, 190

Metestrous bleeding in cow, 228

Metestrus
 - definition of, 140
 - in dog, 366

Metrial gland
 - eosinophilic granular cells in, 22

Metrorrhagia
 - in cow, 22
 - in dog, 365, 378
 - in primates, 22

Milk
 - characteristics in experimentally induced lactation, 565

Milk "let-down" following coitus, 178

Milk production, mammary gland size and, 549

Milk secretion, following prolonged gestation, 521-522

Milk yield
 - effect of
 - anterior pituitary hormones on, 579-582
 - estrogen on, 584-589
 - growth hormone, 579
 - hormones at various lactation stages, 578
 - prolactin on, 581
 - thyroid hormone on, 582-584
 - factors affecting thyroid hormone effect on, 582
 - following artificial induction of lactation, 567-568

Morula, formation of, 420

Mouse
 - estrous cycle of, 144
 - induced ovulation in, 82

Mucus, vaginal
 - chemical composition in cow, 243
 - cyclic changes in
 - cow, 242
 - mare, 281

Mucus
 - effect of hormones in cow on, 499

- Mullerian ducts, 4, 11, 13, 15, 17, 30, 31
 Mullerian tubercle, 11
 Myometrium
 bovine, 21
 cyclic changes in cow, 238
 in man, 21
 in the rat, 21

N

- Nervous system
 centers controlling sexual act, 204
 effect on
 lactation, 555-559
 parturition, 214-215
 gonadotropin secretion control by, 186-203
 humoral transmission to anterior pituitary, 194-196
 lactation control by, 215-216
 nerve fibers controlling anterior pituitary, 192-194
 ovarian hormone released following afferent stimulation, 199
 pituitary secretion control, 192-196
 Neural factors
 influencing parturition, 527
 Neurohypophysis, effect on lactation, 559
 Neurohumoral agents, effect on pituitary, 256
 Nidation
 blastocyst as stimulus for, 473
 time of, in cow, 499
 Nitrogen retention
 effect of androgens on, 115
 Nuclear chromatin body, 3
 use in diagnosing the genetic sex, 1
 Nursing
 effect on
 prolactin content of pituitary, 552
 Nutrition
 effect on
 breeding efficiency in mares, 275
 estrous cycle in dog, 384
 gestation length, 515-510
 sexual season in ewe, 292
 endocrine imbalance due to insufficiency, 516
 of oocyte, 401
 parturition caused by insufficiency of, 516

- Nymphomania
 in dog, 366
 estrogen injection as cause of, 577

O

- Oocyte
 chromosome number in, 401
 maturation of, 401-404
 nutrition of, 401
 secondary, 402
 size of mammalian, 142
 structure of, 400
 Oogenesis in dog, 373
 Os penis
 in Carnivora, 41
 in Chiroptera, 41
 in Marsupialia, 51
 in primates, 51
 in Rodentia, 51
 Ova, *see* Ovum
 Ovarian burst in dog, 369
 Ovarian follicle
 atresia in cow, 231
 chemical composition of fluid in cow, 231
 glycogen in, 233
 growth of, 142
 in cow, 232
 in ewe, 297
 maturation of, 6
 maturation in dog, 372
 regression in mare, 278
 structure of, 400
 Ovarian tissue, survival after freezing, 20
 Ovariectomy
 age and, 129
 effects of, 129-130
 on anterior pituitary, 130
 in birds, 130
 in mammals, 130
 on pregnancy in goat, 500
 Ovary, *see also* under individual species
 age changes in dog, 374
 agenesis of, 1
 alkaline phosphatase in, 20
 anatomy after birth, 18-20
 anatomy of
 in dog, 369
 in mare, 277-278
 anestrous condition in mare, 278

- ascorbic acid in, 20
 compensatory hypertrophy of, 20
 cyclic changes
 in chemical composition in sow, 343-344
 in cow, 231-234
 in dog, 372-370
 in mare, 279-280
 in sow, 311-345
 cystic, causing nymphomania in dogs, 366
 dependence of accessory sex organs upon, 00
 dispensability of, during pregnancy, 84
 dysgenesis, 1
 effect of
 androgens on, 126
 equine gonadotropin during pregnancy on, 497
 estrogen on, 132
 estrogen in mare on, 271
 progesterone on, 138
 removal, on estrous cycle in dog, 366
 on pregnancy, 474
 estrogen content in sow, 344
 gonadal blastema of, 2
 granulosa cells of, 4
 histochemical studies of, 20
 hormone content in sow, 344-345
 interstitial cells of, 8
 lymphatic drainage in human, 20
 medullary sex cords, 9
 palpation in mare, 278
 pregnancy induced changes in, 471-475
 prepuberal development
 in dog, 362
 in sow, 336
 primordial follicles, 5
 primordium of, 2
 progestin content in sow, 344-345
 relative activity between right and left in cow, 230
 relaxin content
 in pregnant sow, 500
 in sow, 345
 secondary sex cords of, 3
 in sow, 341-345
 transplantation after freezing, 20
- Ovary, human**
 decidua-like tissue in, 20
- Oviduct**
 alkaline phosphatase in, 21
 cells of Feyrter in, 21
 chemical composition of fluid in cow, 243
 chemical constituents in, 430
 cyclic changes, 21
 in cow, 234
 in dog, 377
 in ewe, 298
 development of, 11-13
 of dog, 369-370
 epithelium of, 21
 estrogen and, 132
 motility of, in sow, 340
 musculature of, 20
 rate of passage of ova through, in sow, 316
 transport of zygote, 428
- Ovulation, 142**
 control of, in ewe, 309-312
 in cow, 232, 254
 in dog, 373
 in ewe, 290-298
 drugs blocking copulation-induced, 200-201
 effect of
 atropine in cow on, 255
 gonadotropin in mare on, 286
 oxytocin in cow on, 258
 hormonal control in ewe, 325-328
 hormonal factors necessary for, 81-83, 472
 induction by hypothalamic stimulation, 199
 induction in
 farm animals, 81-82
 monkeys by homologous pituitary preparations, 78
 several types of rodents, 82-83
 mechanism of, 404
 in cow, 259
 in mare, 278
 multiple, in mare, 280
 neural control of, in ewe, 310
 neurohumoral mechanisms in cow, 255-258
 progesterone block in ewe, 326-327

- in sow, 342, 349-352
 - time of, 405
 - in cow, 226
 - in dog, 373
 - in mare, 279-280
 - Ovulation fossa
 - mare, 278
 - Ovulation rate
 - breed differences in sow, 350
 - effect of
 - "flushing" in sow on, 350-351
 - inbreeding in sow on, 350
 - nutritional deficiencies in sow on, 352
 - sexual age in sow on, 350
 - in sow, 350-352
 - Ovum
 - activation following fertilization, 414
 - age and number shed in ewe, 297-298
 - external migration of, 406
 - fertilization and development of, 399-432
 - formation from mesothelium, 5
 - incomplete maturation of, 417
 - migration through oviduct in dog, 360
 - multinuclear, 6
 - production after birth, 18-19
 - size in mammals, 401
 - spermatozoon penetration, 412
 - supplementary spermatozoa in rabbit and, 413
 - transplantation of, 81
 - transport of, 405
 - in cow, 230
 - in ewe, 298
 - in sow, 346
 - viability of, 406
 - in dog, 374
 - in mare, 286
 - wastage of, 142
 - Oxytocin, *see also* Pituitrin
 - in blood after stimulation of vulva, 179
 - effect on
 - estrous cycle in cow, 253, 258
 - 17-ketosteroid excretion, 258
 - ovulation in cow, 258
 - prolactin content of pituitary, 553
 - spermatozoon transport, 407
 - uterine contractions, 178
 - formation in placenta, 531
 - labor induced with, in sow, 531
 - in milk release control, 215
 - prolactin release stimulated by, 202
 - reflex release during labor, 527
 - in relation to artificial insemination, 179
 - in reproductive processes, 177-180
 - role in
 - initiating parturition, 489
 - parturition, 530
 - seminal fluid transport, 178-180
 - suckling as release stimulus, 215-216
 - uterine sensitivity at term to, 214
 - Oxytocinase
 - in prolonged labor, 531
- P**
- PMS, *see* Gonadotropin, equine
 - Pancreas, effect on lactation, 554
 - Parabiosis
 - effect on pregnancy in rats, 528
 - intoxication due to incompatibility, 528
 - Parathyroid
 - effect on lactation, 555
 - role in pregnancy, 478
 - Parthenogenesis, 427
 - Parturition, 522-533
 - in dog, 383
 - effect of
 - fetal pituitary on, 531
 - hypothalamus on, 214-215
 - posterior pituitary on, 214-215
 - endocrine factors initiating, 528-533
 - influence of estrogens on, 529
 - initiation of, 526-533
 - hormone decrease and, 528
 - influence of physical factors, 533
 - "insufficiency of fetal nutrition theory", 516
 - mechanisms controlling lactation at, 559-565
 - motility patterns of uterus preceding, 530
 - neural control of uterine muscle, 214-215
 - neural factors influencing, 214-215, 527
 - oxytocinase in prolonged labor, 531
 - progesterone levels as related to, 528

- progesterone-estrogen ratios and, 530
 role of
 hypothalamus in, 531
 oxytocin in, 489-530
 relaxin in, 532
 time of estrus in relation to, in sow, 340
- Penis**
 anatomy of, 49-54
 effect of androgen on, 122
 erectile tissue of, 49
 sigmoid flexure of, 52
 vascular supply to, 52-54
- Perivitelline space**, 401
- Photoperiodism**
 eye as receptor, 188-189
 lack of, in tropical animals, 187
 refractoriness, effect on, 190
- Pineal gland**
 tumors inducing precocious puberty, 212
- Pituitary**
 neural control of secretion, 192-196
- Pituitary ablation**, *see* Hypophysectomy
- Pituitary, anterior**
 acidophils of, 249
 cyclic changes
 in cow, 247
 in gonadotropin content in sow, 348-349
 in sow, 347-349
 delta cells in cow, 247-249, 256
 dependence of
 body growth on, 60
 gonads upon, 60-67
 other glands upon, 60
 effect of
 castration on, 122
 estrogens on, 117
 estrogens in mare on, 286
 ovariectomy on, 138
 oxytocin on prolactin content of, 553-554
 progesterone on, 138
 steroid hormones on prolactin content of, 550-551, 553
 function during pregnancy, 83-84
 gonadotropic activity in mare, 271
 gonadotropin content
 during pregnancy, 476
 in sow, 336-337
- milk yield affected by, 579-582
 nerve fibers as controlling factor, 192-194
 neurohumoral agents controlling, 194-196
 pregnancy cell of, 475
 prolactin content
 during gestation, 551
 as influenced by nursing, 552
 during pseudopregnancy, 551
 role in pregnancy, 475-478
 size during pregnancy, 475
 in sow, 347-349
 stalk section effect on, 194-195
 stimulation by gonadotropins, 105
 transplantation effect on gonads, 195-196
- Pituitary, fetal**
 effect on parturition, 531
- Pituitary gonadotropic complex: FSH and LSH (LH)**, 67-75
- Pituitary gonadotropins**, *see* Gonadotropins, pituitary and individual hormones
- Pituitary, posterior**
 effect on
 lactation, 558
 parturition, 214-215
 hormones secreted by hypothalamus, 257
 role in pregnancy, 479
- Pituitary stalk**, effect of severing on anterior pituitary, 194-195
- Pituitrin**, effect on uterine motility in sow, 346
- Placenta**
 adrenal cortical steroids in, 492
 adrenocorticotropin secretion by, 493
 biochemical changes at term, 526
 chorioallantoic, 451
 classification of, 435
 cotyledons of, 457
 definition of, 434
 degeneration in late pregnancy, 525
 development of, in mare, 454
 effect on mammary development, 546
 endocrine functions of, 491-494
 endometrial cup formation, 497
 endotheliochorial, 459-461
 formation of, 459

- epitheliochorial, 453-456
- estrogen secretion by, 492
- formation of, in dog, 459
- gonadotropin activity in, 493
- hematoma in, 460
- labyrinth of, 460
- maturation in dog of, 460
- morphological divisions in mare, 455
- oxytocin in, 531
- progesterone metabolites in, 492
- regional differentiation in carnivora, 437
- relaxin content in rabbits, 493
- syndesmochorial, 456-459
- tissue relationships of, 452
- "uteroverdin" pigment in dogs, 460
- Placentome
 - in cow, 449
 - definition of, 457
- Polar bodies, formation of, 402
- Polyspermy, in mammals, 409
- Posterior pituitary, *see* Pituitary, posterior
- Posterior pituitary hormones, secretion by hypothalamic nuclei, 257
- Postpartum
 - involution of uterus during, 525
- Postpartum estrus, in sow, 340
- Postweaning estrus, in sow, 340
- Pregnancy, *see also* Gestation and Gestation length
 - adrenal cortical steroids during, 486
 - ameliorating effect on adrenalectomy, 478-479
 - definition of, 470
 - effect of
 - adrenalectomy on, 479
 - corpus luteum removal on, 499
 - estrogen on, 472
 - hypophysectomy on, 474, 477
 - ovariectomy on, 474, 500
 - parabiosis in rats on, 528
 - placenta on, 474-475
 - endocrine mechanisms during, 469-507
 - estrogen titers during, 482
 - function of estrogens during, 475
 - hormonal factors necessary for, 83-84
 - hormone levels during, 479-489
 - maintenance of
 - after ovariectomy in ewe, 501
 - following ovariectomy, 474
 - mammary glands in, 490
 - maternal endocrine functions, 471-491
 - ovaries during, 471-475
 - pituitary hormone content during, 476
 - pituitary size during, 475
 - pregnanediol excretion during, 483
 - progesterone and, 138-139
 - progesterone concentration in blood, 485
 - relaxin in, 474
 - role of
 - anterior pituitary in, 475-478
 - parathyroid during, 47
 - thyroid in, 478
 - steroid excretion, in sheep, 501
 - steroid hormone levels during, 480-487
 - uterine growth during, 489
- Pregnancy diagnosis
 - in mares, 89-90
 - in women, 89-90
- Pregnanediol, 139
- urinary excretion during pregnancy, 483
- Pregnant mare serum, *see* Gonadotropin, equine
- Pregnenolone in testes, 34
- Prepuce, anatomy of, 54-55
- Prepuberal development in sow, 336-337
- Preputial glands, effect of androgen on, 124
- Primary follicle, cow, 231
- Primary sex cells, 2
- Primary sex cords, 2, 3
- Primates, anatomy of uterus, 21
- Proestrus
 - definition of, 140
 - manifestations in dog, 365
- Progesterone, 118-119, 137-140, *see also* Gonadal hormones
 - assay of, 139-140
 - blockage of ovulation in ewe with, 326-327
 - blood levels
 - in cow, 254
 - in ewe, 118, 308
 - after injection, 118
 - during menstrual cycle, 118
 - in peripheral circulation following injection, 118

- in pregnant sheep, 501
- in pregnant women, 118
- prior to and at parturition, 528
- in rabbit, 118
- chemical structure, 113
- control of pituitary by, 70
- decidua reaction and, 137
- effect on
 - estrous cycle in cow, 250
 - in dog, 390
 - in sow, 353-354
 - fertility of cow, 251
 - mammary gland, 139
 - ovary, 138
 - phosphatase activity in cow uterus, 245
 - pituitary gland, 138
 - uterus, 137
- estrogen ratio in parturition, 530
- estrus produced by, 205
- functions of, 473
- induction of estrus in ewes with, 313
- interaction with estrogen, 137
- metabolic effects of, 118
- organs producing, 114
- placental secretion of, 492
- in pregnancy, 138-139
- prolonged pregnancy and, 139
- psychic estrus and, 138
- requirements during pregnancy, 83
- role in female, 137-139
- secretion during estrous cycle of cow, 500
- secretion by follicle, 233
- synergism with estrogen in dog, 387
- titers in blood during pregnancy, 485
- Progestins
 - 20-hydroxypregnenone in sheep, 529
 - in sow ovary, 344-345
 - structure of, 113
- Progestogens, *see also* individual compounds and Progestins
- Progonadotropins, 77-78
- Prolactin
 - control of secretion, 201-202
 - effect on
 - mammary growth, 544
 - milk yield, 581
 - estrogens and pituitary content of, 549
 - estrous cycle and, 141
 - initiation and maintenance of lactation by, 547
- pituitary content
 - nursing stimulus and, 552
 - oxytocin and, 553
 - physiological factors and, 548
 - pregnancy and, 477, 551
 - pseudopregnancy and, 551
 - steroid hormones and, 550, 553
- prolongation of life of corpora lutea by, 74
- release stimulated by oxytocin, 202
- source of, 547
- specific action on mammary alveolar cells, 548
- as third gonadotropin, 74-75
- witches milk and, 489
- Prolonged gestation, 519-522
 - in cattle, 520
 - influence on size of fetus, 521
 - inheritance of, 520
 - labor in, 521
 - in mare, 520
 - milk secretion following, 521-522
 - pathology of
 - in Guernseys, 521
 - in Holsteins, 520
- Pronucleus
 - formation of male and female, 414
 - synthesis of DNA by, 415
- Prostate
 - anatomy of, 42-47
 - development of, 30
 - effect of androgen on, 123
 - enlargement in dog, 47
- Prostatic utricle, 17
- Protein anabolism, effect of estrogen in ruminant on, 116
- Pseudoestrus, in mare, 269
- Pseudohermaphroditism, 12, 15
- Pseudohypophysectomy, 86
- Pseudopregnancy, 141
 - clinical aspects in dog, 381-384
 - effect on uterine infection in dog, 391
 - induction of, 473
 - mammary development in dog during, 383
 - pituitary prolactin content during, 551
- Puberty
 - age of cow at, 224

- in dogs, 362
- effect of
 - inbreeding in sow on, 337-338
 - nutrition
 - in cow on, 224
 - in sow on, 338
- fertility and, 129
- gonadotropic hormone induction of, 213
- neural regulation of onset, 211-214
- ovigenesis in relation to, 19
- precocious, due to brain damage, 211-212
- in relation to season in sow, 338
- role of estrogen at, 129
- in sow, 337-338

R

- Rabbit
 - estrous cycle of, 144
 - gestation length, breed differences in, 513
 - induced ovulation in, 82

Rat

- anatomy of uterus, 21
- effect of
 - androgen on perineal muscle, 122
 - fetal hypophysectomy in, 16
 - parabiosis on pregnancy in, 528
- estrous cycle of, 144
- hypophysectomy during pregnancy, 83-84
- hypophysectomy and replacement therapy, 60-67
- induced ovulation in, 82
- metrial gland of, 22
- response to gonadotropins in, 66-67
- Refractoriness, photoperiodism affected by, 190
- Relaxin, 22, 146-147
 - assay of, 147
 - blood titers during pregnancy, 489
 - chemical nature of, 146
 - content in sow ovary, 345
 - effect on
 - cervix of cow, 240, 500
 - mammary gland of ewe, 501
 - effect in sow, 355
 - estrogen and, 140
 - functions of, 474
 - mammary gland growth and, 147

- organs containing, 146
- ovarian content in pregnant sow, 500
- physiological actions, 146
- in placenta of rabbits, 493
- progesterone and, 147
- role in parturition, 532
- sites of formation, 532
- synergism with estrogen, 532

Reproduction

- adrenal hormones and, 172-177
- dietary-hormonal interrelations in, 86-88
- effect of
 - afferent stimuli from genital tract, 192
 - endemic goiter on, 157
 - temperature on, 190-191
 - thyroid hormone on, 584
- gonadal hormones and, 111-154
- oxytocin and, 177-180
- pituitary gonadotropins and, 59-110
- role of
 - hypothalamus in, 188-191
 - thyroid hormones and, 157-172
 - visual contact as a component, 191
- Reproductive cycle, light as controlling factor, 186-191
- Reproductive organs
 - anatomy after birth, 18-23
 - of fetal dog, 361
 - size in cow, 230
- Rete cords, 2
- Rete ovarii, in dog, 376
- Rete testis, anatomy of, 36

S

- Schweigger-Seidel technique, isolation of uterine glands by, 21
- Scrotum
 - anatomy of, 40-41
 - blood vessels and nerves of, 40
 - effect of androgen on, 122
 - external cremaster muscle of, 40
 - fascia of, 40
 - tunica dartos of, 40
- Season
 - effect on gestation length
 - in cow, 514
 - in ewe, 515
 - in goat, 515
 - effect on implantation of fetus, 514

- Secretion of pituitary gonadotropins,
regulation of, 78-80
- Semen
of dog, 392
effect of estrogens on, 133
site of deposition, 406
volume of ejaculate in various species,
406
- Seminal vesicles
anatomy of, 47
of boar, 47
development of, 30
of ruminants, 47
of stallion, 47
- Seminiferous tubules
effect of androgen on, 125
length of, 32
- Sertoli cells, 33, 69
histochemical studies of, 34
tumors of, 34
- Sex differentiation, role of developing
gonads on, 13-18
- Sex drive, effect of androgens in female,
126
- Sex hormones, *see* individual compounds
- Sex reversal
effect of
androgens on, 127
hormones on, 495
- Sex skin, effect of estrogens on, 116
- Sexual behavior, in dog, 365-366
- Sexual maturity, definition of, 119
- Sexual season, *see also* Breeding season
in ewe, 292
influence of psychic factors on, 294
- "Silent" estrus
in ewe, 294
in mare, 269, 285
in sow, 339
- Sinovaginal bulbs, 12
- Somatotropin, *see* Growth hormone
- Sow
activity during estrous cycle, 349
anterior pituitary of, 336-337, 347-349
corpus luteum of, 342-343
cyclic changes
in anterior pituitary, 347
in genital organs, 341-349
in ovary, 341-344
in oviduct, 345
in uterus, 345
in vulva during estrous cycle, 349
duration of estrus in, 339-340
effect of
adrenaline on uterine motility, 346
estrogens on estrous cycle of, 354-355
"flushing" in, 350-351
gonadal hormones in, 353-355
gonadotropins in, 352-353
nutritional deficiencies on ovulation
rate, 352
pituirrin on uterus, 346
progesterone on estrous cycle of,
353-354
relaxin, 355
estrogen content in ovary of, 344
estrous cycle of, 335-357
experimental modification of estrous
cycle, 352-355
gestation length
paternal influence on, 513
relation to age of dam, 519
gonadotropin content of pituitary, 348-349
hormone content of ovary, 344-345
induced ovulation in, 82
labor induced with oxytocin in, 531
motility of oviduct in, 346
ovarian development in, 336
ovary of, 341-345
ovulation in, 342, 349-352
postpartum estrus in, 340
postweaning estrus in, 340
prepuberal development of, 336-337
progesterin content of ovary, 344-345
puberty of, 337-338
relaxin content of ovary, 345
"silent" estrus of, 339
time of estrus in relation to parturi-
tion, 340
transport of ova through oviduct, 346
uterine motility during estrous cycle,
346
uterus of, 345-346
vagina of, 346-347
- Species specificity, of pituitary gonado-
tropins, 77-78
- Spermatic artery, branches to epididymis,
35

Spermatic cord, 35
 Spermatogenesis, 33
 Spermatozoa
 capacitation of, 408
 capacitation in cow, 227
 effect of
 oxytocin on transport, 407
 uterine contractions on transport, 407
 fertile life of, 408
 hyaluronidase in, 411
 number at fertilization site, 408
 number in ejaculate, 408
 penetration through
 cumulus oöphorus, 411
 vitellus, 412
 zona pellucida, 411
 perforatorium of, 412
 pressure of fluid and movement of, 37
 rate of transport in female, 407
 supplementary, penetrating zona pellucida, 413
 transport of
 in uterus, 407
 in ewe, 299
 viability in tract of mare, 286
 zona lysis of, 412
 Stallion
 prostate of, 42-43
 Sterility
 and estrogen in male, 133
 Steroid hormones, *see also* individual compounds
 effect on
 gonadotropin production, 78
 prolactin content of pituitary, 553
 sources of, 114-115
 Steroid nucleus
 nomenclature of, 112
 Stilbestrol, effect on vagina of cow, 246
 Suckling, effect upon maintenance of lactation, 555
 Superovulation, 81
 in cow, 252
 in ewe, 326
 Synergism
 between gonadotropins, 72-74
 non specific, 72
 Syngamy, 414

T

Temperature, reproduction affected by, 186-187, 190-191
 Testis
 anatomy of, 31-36
 biosynthesis of testosterone by, 114
 direct effect of androgens on, 125
 effect of androgen on, 124
 elevation of, 40, 41
 internal spermatic artery of horse fetus and, 34
 interstitial cells of, 2
 light as stimulus to growth of, 187
 mediastinum of, 31
 pituitary changes due to, 202-203
 production of estrogen in stallion by, 72
 reciprocal action on pituitary, 115
 seminiferous tubules of, 32
 shape of, in domestic animals, 31
 thermal regulation of, 40
 vascular supply to, 34-36
 venous drainage of, 35
 Testosterone
 biosynthesis by testis, 114
 chemical structure of, 112
 17-ethyl-19-nor-testosterone
 chemical structure of, 113
 Thiouracil
 effect on
 body growth, 162
 ovarian response to gonadotropins, 162
 reproductive organs of male, 162, 165, 171-172
 Thyroid
 effect of
 ambient temperature on, 159-161
 hypo- and hyperthyroidism on response to gonadotropins, 161-162
 removal on lactation, 478
 effect on mammary development, 545
 in female reproduction, 162-164
 in fetus, 496
 interrelationship with adrenal, 177
 in male reproduction, 164-166
 relation to estrous cycle in cow, 250
 role of
 in pregnancy, 478
 in reproductive processes, 157-172

Thyroid hormone

causes of diversity of results with,
169-170

effect on

egg production in chickens, 168-
169, 172

eggshell thickness, 169

milk yield, 582

semen of birds, 166-168

Thyroidectomy

effect on

libido of bull, 165

the male, 165

pregnant animals, 164

influence on estrous cycle in cows, 163

Thyroprotein

effect on

body growth in cockerels, 167-168

egg production and quality, 168-169

growth of mice, 162

the male, 165-166

rabbit, 165

reproduction in cows, 163-164

semen production in cockerels, 167-
168

testes weight in cockerels, 167

effectiveness of, 159

Thyroxine

secretion rate

individual variations in, 158-159

seasonal variations in, 159-160, 164-
165, 170

Trophoblast, 424

Tubuli recti, 36

Tunica albuginea, 31-32, 35

U

Udder congestion relieved by estrogen,
588

Ureter, agenesis of, 11

Urogenital sinus, 11-13, 15, 18, 31

Uterine glands, metestrous atrophy in
dogs of, 378

Uterine milk, relation to nutrition of early
embryo, 430

Uterine motility

effect of

epinephrine on, in cow, 246

sexual stimulation in cow on, 257

nature of, in sow, 346

Uterine tubes, *see* Oviduct

Uterosacral ligaments, 20

Uterovaginal canal, 11, 13

from fusion of Müllerian ducts, 11

Uteroverdin, 460

Uterus

anatomy of, 21, 230, 370

in cow, 230

in dog, 370

chemical composition of fluid in cow,
243

cyclic changes

in cow, 236-238

in dog, 378

in ewe, 299

in mare, 282

nature of, 143

in sow, 345-346

development of, 11-13

effect of

adrenaline on motility in sow, 346

estrogen on, 131

pituiratin on motility in sow, 346

progesterone on, 137

epithelial regeneration of intercaruncu-
lar area, 458

growth in pregnancy, 489

involution of, after parturition, 229,
490, 525

lymphatic drainage of, 23

motility of

preceding parturition, 530

in sow, 346

pressure within, during labor, 524

round ligaments of, 12, 20

spontaneous motility in cow, 245

Uterus masculinus, 30-31

in boar, 31

in bull, 31

in dog, 31

in stallion, 31

uterine glands in, 31

Utriculus, prostatic, 30

V

Vagina

alkaline phosphatase in rat, 23

anatomy in dog, 371

- cyclic changes
 - in cow, 239
 - in dog, 379
 - in mare, 281, 371
 - nature of, 143
 - in sow, 346-347
- cytoplasmic fibrillae of, 23
- development of, 11-13
- pH of secretions in mare, 284
- Vaginal slices, response to estrogens in
 - tissue culture, 117
- Vaginal smear
 - cyclic changes
 - in cow, 241
 - in dog, 379-381
 - in ewe, 300
 - in mare, 283
- Vas deferens, development of, 30
- Vesiculase, 124
- Vitamin E deficiency, seminiferous tubules, degeneration and, 87
- Vitellogenesis
 - definition of, 437
- Vitellus, 400
 - spermatozoon penetration of, 412
- Vulva
 - changes in relation to estrus in sow, 341, 349
 - vestibule of, 13

W

- "Wakefulness"
 - mechanism of photoperiodic stimulation, 189
- Witches milk, prolactin and, 489
- Wolffian duct, 4, 11, 12, 13, 17, 30
- Woman
 - induced ovulation in, 82
 - pregnancy diagnosis in, 89-90

X

- X-irradiation, effect on estrous cycle in
 - dog, 385

Y

- Yolk sac
 - in cat, 441
 - in cow, 440
 - definition of, 438
 - in dog, 441
 - in ewe, 440
 - in mare, 441
 - in sow, 440

Z

- Zimmerman reaction, 129
- Zona pellucida, 400
 - reaction following fertilization, 413
 - spermatozoon penetration of, 411
- Zygote
 - cleavage of, 418